IMPACT OF BIOCHAR ON T. AMAN RICE UNDER LEAD (Pb) STRESS CONDITION

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Dedicated to

My Beloved Parents

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CERTIFICATE

*This is to certify that thesis entitled, **"Impact of Biochar on T. Aman Rice Under Lead (Pb) Stress Condition"** submitted to the faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of**MASTER OF SCIENCE** in**AGRONOMY,** embodies the result of a piece of bona fide research work carried out by **Sabrina Habib,**Registration No.: **13-05743** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been fully acknowledged.

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Abstract

The present study was conducted in net house of Sher-e-Bangla Agricultural University during July to December 2018 to understand the role of biochar under Lead (Pb) stress condition. Different levels of lead chloride($PbCl_2$) (0, 75 and 150 mg kg^{-1}) was applied to understand the effect of Pb on T. amanrice (BRRI Dhan62) and different doses of biochar (0, 2, 4 and 6 t ha^{-1}) was applied for reducing Pb toxicity in rice plant. This experiment was laid out in completely randomized design with four replications. Exposure of Pd decreased growth of *aman* rice by decreasing plant height, tiller number, leaf number, leaf area index, root length and root weight. External application of 75 and 150 mg $kg^{-1}$of $PbCl_2$decreased effective tillers (12 and 25%, respectively), number of grain (8 and 21%, respectively), grain yield (12 and 30%, respectively) and straw yield (4 and 12%, respectively). Addition of different doses of biochar influenced growth and yield attributes. Growth and yield of rice increased with increasing the doses of biochar upto a certain extent (4 t ha^{-1}). Application of 4 t ha^{-1} of biochar in *aman*rice increased grain yield by 15%. However, application of different doses of biochar have no impact on riceunder Pb stress conditions.

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INTRODUCTION

The presence of heavy metals in soil, water and air are global problems that are a growing concern to the environment. After industrial revolution and ever increasing urbanization, there has been a consistent addition of heavy metals (HMs) in the environment, which is now becoming a challenge to produce crops from the contaminated soil. There are several sources of heavy metal contamination in the environment including soils, water and air. Soil contamination, with HMs, is a serious threat that has arisen from various human activities such as mining (Abdelhafez*et al*., 2014), industry,agriculture practices (Abdelhafez*et al*., 2012) or treated wood structures (Abdelhafez*et al*., 2009). In addition, industrial processing, automobiles, use of synthetic fertilizers, and other agro-chemicals are building pools of various heavy metals of which lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), are of major concerns (Huang *et al*., 2007). Heavy metals are one of the most prevalent contaminants causing public health problems, entering the body in food, ingestion of soil and inhalation of dust. The build-up of heavy metal levels in agricultural soils leads to soil contamination and increases heavy metals uptake by growing plants (Abdelhafez*et al*., 2012), which affects food quality and safety.

Heavy metals cause deleterious impacts on human health, soil life, plant metabolism, and other aquatic and terrestrial ecosystems (Grimm *et al*., 2008). Heavy metals in agricultural lands may transfer to consumer's body through soil-plant-food interaction and may cause some serious defects and abnormalities (Chaney *et al*., 2004). Different types of pollutants vary in their specifications, concentrations, and toxicity levels posing a serious threat to agricultural productivity, thus targeting a wide range of crop plants (Arshad*et a*l., 2008). Among various HMs that affect plants, Pb is one of the most hazardous metal and ranked second after arsenic due to its potential of toxicity to plants and human beings as well as occurrence and distribution over the globe (ATSDR, 2003).

Lead is being added and accumulating profoundly in the soil through anthropogenic activities (Alloway, 2013). Being a toxic substance, and having high transfer rates (from soil to plant), it is therefore studied broadly especially in context to food safety, quality, and biotesting purposes (Uzu*et al*., 2009). It adversely affects plant's morpho-physiological and biochemical processes such as seed germination and seedling growth, plant phenology, and root/shoot ratio; disrupts cell membrane permeability, photosynthesis, plant respiratory processes, chlorophyll contents, chloroplastic lamellar organization and cell division; and cause growth and developmental abnormalities as well as ultrastructure changes (Dogan *et al*., 2009; Ling and Hong, 2009; Gupta *et al*., 2009; Maestri*et al*., 2010). However, its effectiveness depends on stress intensity and duration, plant stage of stress exposure, lead concentration, and its bioavailability in plant organs. Plants have their own metal uptake, accumulation, translocation, detoxification, excretion, and compartmentalization mechanisms to respond to Pb toxicity (Jiang and Liu, 2010). There is a wide range of genotypic variation exists among rice plants regarding uptake, translocation, and accumulation of Pb (Cheng *et al*., 2006). A study conducted by Liu et al. (2013) in China confirmed the genotypic specific behavior of rice regarding Pb translocation from roots to shoot with higher translocation factors(TF) in hybrid indica followed by indica and japonica. Rice, a major food crops in many countries all over the world, is now prone to heavy metal toxicity due to recurrent usage of metal-enriched agro-chemicals, frequent use of waste water, and sewage sludge (as organic amendments), which developed pools of HMs within the soil (Fangmin*et al*., 2006). Lead accumulation in rice pronouncedly reduced leaf chlorophyll, protein and nitrogen contents, carotenes, and hill reaction while enhanced various enzymatic activities like catalase, ribonuclease, and acid phosphatase (Chatterjee *et al*., 2004). Moreover, Pb accumulation in rice grains also employ quality issues and adverse health complications (Shraim, 2014). Fangmin*et al*., (2006) collected rice grain samples from different regions of China and found the eminent Pb concentrations (> 0.2 mg kg^{-1}) in rice grains. So, increased Pb contents in rice raised rice quality and food security issues. Soil contamination with Pb and its subsequent translocation and accumulation in rice plants (especially in grains) and its subsequent effects on human health has previously been reported by various researchers (Davis *et al*., 1993; Liu *et al*., 2013); however, further research is being employed now-a-days on affects and speciation ofPb in rice to develop Pb-tolerant strains of rice which can store more Pb contents within the roots and transfer its minimum to the edible part to ensure food quality.

Biochar, a carbon-rich compound, is a product of biowaste prepared under oxygen-limited conditions (pyrolysis) have a great potential to amend the Pb-contaminated soils (Lehmann, 2007). Immobilization of Pb in paddy soils and its hampered accumulation in rice due to biochar application is recently explored (Bian*et al*., 2013; Zhang *et al*., 2013). Due to its high pH, porosity, cation exchange capacity (CEC), and active functional groups, biochar retain Pb to adsorb and allow it to translocate to the plants (Zhang *et al*., 2013). Immobilizing role of CEC in chemically mobile metals under biochar-amended paddy fields are also reported by Uchimiya*et al*., (2011). Some short-term studies on contaminated soils with biochar amendments showed a remarkable reduction in rice lead contents; however, its long-term effects on Pb immobilization and its reaction with biochar are not analytically examined (Zhang *et al*., 2013). Biochar application improved soil structure, productivity, and cycling of some minerals (P, K, and Si) by improving soil pH and soil organic carbon (SOC) contents (Biederman and Harpole, 2012).

Organic molecules, cations, and anions present in the soil can form precipitates by reacting with metallic cations present at the biochar's surface.Moreover, metallic cations have high adherent capacity than anions (Keiluweit*et al*., 2010). Mostly, Pb adheres to clay particles through ionic, covalent, and hydrogen bonding (Bradl, 2004). Based on crop productivity and soil remediation, biochar application relates to plant growth and development, improved soil structure, increased water holding capacity, and enhanced soil microbial activity in Pb-contaminated soils (Sohi *et al*., 2010). However, organic and inorganic amendments, along with biochar application, are required in most of the cases for soil structure improvement and land stabilization (Steiner *et al*., 2008). Biochar application can be beneficial in excessive plant nutrition while detrimental in limited nutrient conditions. However, application of biochar with some organic amendments could be more beneficial for soil remediation. It further maintains a balance between nutrient acquisition and immobilization or exclusion of pollutants including Pb, thus required growth and plant biomass is difficult to attain from contaminated lands (Peltz *et al*., 2010).

Considering the issues, the present piece of research work was carried out with the following objectives:

1) To understand the effect of Pb on aman rice,
2) To understand the role of Biochar on rice, and
3) To know the role of biochar in Pb accumulated soil.

Chapter II

REVIEW OF LITERATURE

There has been increasing contamination of agricultural land by heavy metals. This pollution in soils has increased the stresses on terrestrial ecosystems and societies. Heavy metal pollution in crop lands can result in enhanced dietary exposure through soil–plant–food chain transfer, causing elevated levels of toxic metals in human organs. Of metals produced through anthropogenic activities, Cd and Pb are considered the most harmful for human health and accumulate at highest rates in soils. Organic manure can be a way to mitigate the problem. Biochar is an organic carbon riched material which plays different role to ameliorate the Pb stress. This chapter includes different reviews to Pb stress and role of biochar. But the available relevant reviews are very limited in context to Bangladesh. Some of the recent and past information have been reviewed under the following headlines.

2.1 Abiotic Stress

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. In both natural and agricultural conditions, plants are frequently exposed to environmental stresses. The complex nature of the environment, along with its unpredictable conditions and global climate change, are increasing gradually, which is creating a more adverse situation. A number of abnormal environmental parameters are collectively termed abiotic stress. Plant stress is the adverse reaction of plants to environmental conditions that are unfavorable to growth. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. Plants are frequently exposed to a plethora of unfavorable or even adverse environmental conditions, termed abiotic stresses (such as salinity, drought, heat, cold, flooding, HMs, ozone, UV radiation, etc.) and thus they pose serious threats to the sustainability of crop yield. Abiotic stresses remain the greatest constraint to crop production worldwide. It has been estimated that more than 50% of yield reduction is the direct result of abiotic stresses (Hasanuzzaman *et al*., 2012a). Abiotic stresses change the metabolic processes the plant and alter the growth developmental process, decreased the growth and the procreation of the plant and plant died (Wei and Zhou, 2008).

2.1.1 Heavy metal stress

Environmental pollution has become a key focus of concern for all the nations worldwide, as not only the developing countries but developed nations as well are affected by and suffer from it. Among all the pollutants, heavy metals are most dangerous one as these are nonbiodegradable and persist in environment. There are several sources of heavy metal contamination in the environment including soils, water and air. Soil contaminationwith HM, is a serious threat that has arisen from various human activities such as mining (Abdelhafez*et al*., 2014), industry, agriculture practices (Abdelhafez*et al*., 2012) or treated wood structures (Abdelhafez*et al*., 2009).

The term heavy metal refers to any metallic chemical element that has a relatively high density. The density of heavy metals is usually more than 5.0 gcm^{-3}. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), lead (Pb), copper (Cu), zinc (Zn), cobalt (Co), nickel (Ni), and iron (Fe). These metals are classified in to three categories: toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc). Toxic metals cause toxicity to organisms even at very lower level of concentration(Lide, 1992).

Plants are exposed to HMs contamination from the air, water, soil, and sediments. Higher plants can uptake metals from the atmosphere through shoots and leaves plus entry via roots and rhizomes from the soil (Lyubenova and Schröder, 2010). Toxicity of metals within the plant occurs when metals move from soil to plant roots and get further transported and stored in various sites in the plant (Verma and Dubey, 2003). The extent to which higher plants are able to uptake HMs depends on several factors. These include the concentration of metal ions in the soil and their bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature, and concentration of other elements (Benavides *et al*., 2005). The uptake, translocation, and accumulation of HMs in plants are mediated by an integrated network of physiological, biochemical, and molecular mechanisms and occur at extracellular and intracellular levels of the tissues and organs of plants grown under contaminated sites. Toxicity of heavy metals develop various stress symptoms in plants. Ultimate result is growth inhibition and further the death of plant. Ullah *et al*., (1999) reported higher metal concentrations in soils of different sites around Dhaka, Bangladesh, are of growing concern and there is a strong need for remediation of these sites. Heavy metals show its toxic effects in plants connected with water relations, especially in the early stage of plant growth, it influences membrane transport and inhibit root growth and enzyme activities (Appenroth, 2010). Heavy metals are one of the most prevalent contaminants causing public health problems, entering the body in food, ingestion of soil and inhalation of dust.

2.1.1.1 Lead (Pb) stress

Lead is the most prevalent heavy metal contaminant. Lead occurs naturally in trace quantities, and its average concentration in the Earth's crust is about 20 ppm. Weathering and volcanic emissions account for most of the natural processes that mobilize Pb, but human activities are far more significant in the mobilization of Pb than are natural processes (Wright and Welbourn, 2002). Early uses of Pb included the construction and application of pipes for the collection, transport, and distribution of water. Lead is now the fifth most commonly used metal in the world. It was used in pipes, drains, and soldering materials for many years. Many of the compounds of Pb are rather insoluble, and most of the metal discharged into water partitions rather rapidly into the suspended and bed sediments. Here, it represents a long-term reservoir that may affect sediment-dwelling organisms and may enter the food chain from this route (Wright and Welbourn, 2002). Being a toxic substance, and having high transfer rates (from soil to plant), it is therefore studied broadly especially in context to food safety, quality, and biotesting purposes (Uzu*et al*., 2009). It adversely affects plant's morpho-physiological and biochemical processes such as seed germination and seedling growth, plant phenology, and root/shoot ratio; disrupts cell membrane permeability, photosynthesis, plant respiratory processes, chlorophyll contents, chloroplastic lamellar organization and cell division; and cause growth and developmental abnormalities as well as ultrastructure changes (Dogan *et al*. 2009; Ling and Hong, 2009; Gupta *et al*., 2009; Maestri*et al*., 2010). It further results in reactive oxygen species (ROS) production such as superoxide radicals (O•−2), hydroxyl radicals (OH•), and hydrogen peroxide (H_2O_2) that react with micro and macro cellular organelles to cause cell damage (Reddy *et al*., 2005). However, its effectiveness depends on stress intensity and duration, plant stage of stress exposure, lead concentration, and its bioavailability in plant organs. Plants have their own metal uptake, accumulation, translocation, detoxification, excretion, and compartmentalization mechanisms to respond lead toxicity (Jiang and Liu, 2010).

Liu *et al*. (2010) showed that a soil Pb concentration of 800 mg kg^{-1} is moderately toxic to rice with 10–30% decreases in plant biomasses. A soil Pb concentration of 2000 mg kg^{-1} is a soil Pb level that severely inhibits rice growth with 20–50% decreases in plant biomasses and significant reductions in grain yields. They also concluded that the genotype indica was generally more sensitive to soil Pb stress than the genotype japonica, especially at earlier growth stages (such as at the tillering stage), and in grain yield. Phang *et al*. (2011) concluded that Pb exposure to rice seedlings showed a more than 50% inhibition of root growth, which was associated with increased free-radical production by cell wall–accumulated Pb. Lamhamdi*et al*. (2011) observed a significant reduction in seed germination and seedling growth in wheat plants exposed to Pb. They found 98% germination under control conditions; whereas it was only 71% in the treated seeds at a concentration of 3 mM Pb. Lead exposure also markedly reduced the biomass of seed, root, and shoot growth of the seedlings. Chatterjee *et al*. (2004) said that 1 mM of Pb $(NO3)_2$ decreases 19.51% yield of *Oryza sativa*. Liu *et al*. (2003) also said that 800 mg kg^{-1} Pb-COOH reduces 24.53% yield of *Oryza sativa* (*Japonica*) cv. Wu yujing No. 3.

2.2 Biochar

Biochar is pyrolyzed (charred) biomass, or also commonly known as charcoal or agrichar, produced by an exothermic process called pyrolysis (Lehmann and Joseph, 2009). Pyrolysis is the combustion of organic materials in the presence of little or no oxygen, leading to the formation of carbon-rich char that is highly resistant to decomposition (Thies and Rillig, 2009). As a result , biochar can persist in soils and sediments for many centuries (Downie *et al*., 2011), and has great potential to improve agronomic production when applied as a soil amendment. Biochar have high chemical stability in contaminated soils and is a C-rich material. A lot of researches are initiated to explore the distinctive use of biochar for continuing C sequestration (Houben *et al*., 2013). Biochar which is porous and has high C content is prepared by pyrolysis of organic waste (Houben *et al*., 2013). Carter *et al*. (2013) defined biochar asit is a porous carbonaceous solid material manufactured by the process of thermo-chemical decomposition under little supply of oxygen appropriate for the benign and continuing storage of carbon. The International Biochar Initiative (IBI) described biochar as a charcoal which is used as a tool for agricultural and environmental management (Xu D *et al*., 2014). Biochar has proved to be a very effective tool for treatment of contaminated soils due to these reasons: it effectively adsorbs HMs and decreases bioavailability and toxin-induced stress to plants and microorganisms. Biochar compounds are a good source of organic material and mineral nutrients for microbes. It promotes the beneficial microbes that promote remediation and protect them from predators (Rutigliano*et al*., 2014). Biochar improves the soil fertility and plant growth by improving physical and chemical properties of soil and also increases the availability of useful nutrients (Houben *et al*., 2013). In soils the use of biochar has proved to raise the stable C pool and minimize the increasing concentration of atmospheric CO_2 (Fang*et al*.,2015). Several studies have shown that biochar can improve physicochemical and biological soil properties and plant growth (Abdelhafez*et al.*,2014). Therefore, it was shown that biochar has the potential to improve N fertilizer use efficiency of plants (Chan *et al*., 2007; Ding *et al*., 2010; Gaskin *et al*., 2008). Plant nutrient uptake and availability of elements such as P, K and Ca are typically increased, while free Al in solution is decreased in solution in biochar-amended soils. This occurs as a function of biocharhigh porosity and surface to volume ratio, together with an increase the in the pH of acid soils, attributed to the basic compounds found in biochar (Chan *et al*., 2007).

2.2.1 Impact of biochar under stress condition

Abiotic stresses like heavy metals induced stress can be demonstrated in term of dry biomass because dry biomass is the total outcome of different characteristics. Biochar application improves that dry biomass that indicates reduction in metals induced stress which is achieved by biochar that immobilized the metals in soil. According to Beesley and Marmiroli., (2011) sorption is the mechanism through which we can immobilize these toxic metals by biochar addition. Bilgic and Caliskan., (2001) suggested that π-electrons play a vital role in the immobilization when biochar is applied. These π-electrons are part of aromatic functional groups like –OH,–COOH and C=N. Uchimiya*et al*. (2010) suggested the semi sorption of surface by electrons in assistance with π-electrons that is major cause of heavy metals mobility reduction due to biochar addition. Similar trend of metals intake was observed byPark *et al*. (2011) where they successfully immobilized the metals using sludge biochar as where pollutary waste biochar significantly decreased the Pb and Cd intake in *Brassica campestris*.

Shen *et al*.(2016a) applied a hardwood biochar to a sandy contaminated soil (95% sand) and observed a significant decrease in leached nickel and zinc from the soil 3 years after the treatment. In contrast, they applied the same biochar to a lead-contaminated kaolin (35% clay) (Shen *et al*., 2016b), and the treatment showed no significant influence on the lead mobility in the soil.

Cui *et al*. (2012) reported that biochar increased soil pH and SOM, and significantly reduced metal bioavailability, leading to a decrease in rice and wheat grain metal concentrations over the short-term (i.e., 2 years) . Cui *et al*. (2016) showed a similar long-term (i.e., 5 years) trend with wheat straw biochar-Cd/Pb sorption in an amended paddy soil.

2.2.2 Lead(Pb) remediation by biochar

Namgay*et al*. (2010) documented a decrease in the accessibility of heavy metals after the contaminated soil which was amended with biochar, due to reduction of absorption of the heavy metals is reduced. Unlike many other biological amendments, biochar having the ability to increase soil pH. Benavides *et al*.(2005) might have improved sorption of these metals, consequently decreasing their bioavailability for plant uptake.

Abdel Hafez *et al*.(2014)showed that the addition of biochar to shooting range soil significantly increased the water- soluble form of Pb; however, the exchangeable form was sharply decreased. Therefore, the available form of Pb was decreased by increasing the rate of added biochar. Clearly, the addition of biochar decreased the exchangeable form of Pb, and the highest reduction was observed at the highest application rate of biochar(10%).

Chapter III

MATERIALS AND METHODS

This chapter illustrates the concerning methodology used in execution of the experiment to study the impact of biochar on aman rice under Pb stress condition.This part comprises a briefdescription of locations of experimental site, planting materials, climate and soil, seedbed preparation, layout and design of the experiment, pot preparation, fertilizing, transplanting of seedlings, intercultural operations, harvesting, data recording procedure, statistical analysis etc. which are presented as follows:

3.1 Experimental site

This experiment was conducted in the Sher-e-Bangla Agricultural University farm, Dhaka-1207, Bangladesh during T. aman season of 2018. Location of the site is 23°74' N latitude and 90°35' E longitude with an elevation of 8 meter from sea level (Islam, 2014; Laylin, 2014) in Agro-ecological zone of "Madhupur Tract" (AEZ-28). The experimental site is shown in the map of AEZ of Bangladesh (Appendix 1).

3.2 Soil characteristics

The soil belonged to The Modhupur Tract, AEZ – 28 . Top soil was silty clay in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. Soil pH was 5.6 and had organic carbon 0.73%. The experimental area was flat having available irrigation and drainage system and above flood level.

3.3 Climate condition

The geographical location of the experimental site was under the subtropical climate, characterized by three distinct seasons, winter season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October (Edris *et al*., 1979) (Appendix II).

3.4 Experimental details

3.4.1 Treatments and factor of the experiment

Factor A: Lead (Pb) concentration

1. $PbCl_2$ 0 mg kg^{-1} soil (Pb_0)

2. $PbCl_2$ 75 mg kg^{-1} soil (Pb_1)

3. $PbCl_2$ 150 mg kg^{-1} soil (Pb_2)

Factor B: Biochar management

1. Control (B_0)
2. Biochar 2 t ha^{-1} (B_1)
3. Biochar 4 t ha^{-1} (B_2)
4. Biochar 6 t ha^{-1} (B_3)

3.4.2 Experimental design and layout

The experiment was laid out in completely randomized designwith four replications Each replication is sub divided into 12 unit of pot. The treatments were randomly distributed to the unit pots with each replication.

3.5 Growing of crops

3.5.1 Seed collection

BRRI dhan62, a high yielding variety of rice, was used as a test crop. The variety was developed by the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, as a short duration T. amanrice. BRRI dhan62 is a zinc enriched rice variety with 19 $mgkg^{-1}$ Zinc and 9% protein. The life duration of this rice variety is 100 days and yields about 3.5-4.5 tha^{-1}.

3.5.2 Raising of seedling

Seeds were washed several times with fresh water and soaked in a dark place for about 48 hours. The uniformly germinated seeds were then transferred to the nursery bed and were took about 25 days to produce seedling.

3.5.3 Pot preparation

Soil were collected from the field and exposed to the sun for 12 hours. Soils were mixed and turned twice a day for uniform drying. The soils were then be cleared of rocks, coarse organic materials, weeds, stubbles etc. 48 pots were taken for the experiment. Size of the pot was 14″ height and 30cm diameter. About 10kg soil were taken for each pot. Required amount of Organic manures and chemical fertilizers were added to each pot. Experimental pots were laid down as per treatment and design.

3.5.4 Application of fertilizers and manure

All the fertilizers, except urea were applied at final land preparation. Urea was applied in two equal splits. The first split was applied during transplanting of seedling, the second split after 30 days of transplanting i.e. at active vegetative stage. The fertilizer was thoroughly mixed with the soil by hand.

Table 1: Sources and rates of different organic & chemical fertilizer

Fertilizer	Rate
Cowdung	5 (tha^{-1})
Urea	9 ($gpot^{-1}$)
TSP	8 ($gpot^{-1}$)
MP	8 ($gpot^{-1}$)
Gypsum	8($gpot^{-1}$)

3.6 Transplanting of seedling

Seedling of 25 days old were transplanted into the pots on 9th August 2018. The seedlings were carefully uprooted from the field. There were three hills per pot and one seedling was used per hill.

3.7 Intercultural operations

The following intercultural operations were done for ensuring the normal growth of the crop. Top dressing of urea was done as per schedule and the normal cultural practices including weeding and insecticides spray were followed as and when necessary. There were some incidence of insects especially for rice stem borer, rice bug, which was controlled by spraying Diazinon 50EC. Irrigation was also done as per requirement.

3.8 Data collection and sampling procedure

3.8.1 Growthparameter

The data of growth contributing characters of crop were calculated as follows:

1. Plant height
2. No. of tiller
3. No. of leaves
4. Leaf area index
5. Root length
6. Root weight

3.8.1.1 Plant height

The plant height was measured from the ground level to the top of the panicle. Plants of 3 hills were measured and averaged for each pot. Plant height data was taken at 20, 40 and 60 DAT and at harvest time.

3.8.1.2 Number of tillers per hill

Number of tiller were counted from 3 hills of each pot and their average was calculated as well.

3.8.1.3 Number of leaves per hill

Number of leaves were counted from 3 hills of each pot and their average was also calculated.

3.8.1.4 Leaf area index

Length and breadth of a leaf from each hills following by 3 hills from each pot were calculated. In this way LAI were calculated by using the following equation-

$$LAI = \frac{Surface\ area\ of\ leaf\ sample\ (m2)}{Ground\ area\ from\ where\ the\ sample\ was\ collected\ (m2)}$$

3.8.1.5 Root length

After harvesting, root stalk were collected and washed several times to clean the dirt and soil from the root. Then root length was calculated from the collar region in a wet condition.

3.8.1.6 Root weight

Roots from each pot were collected carefully. Washed them several times with fresh water and dried. After that they were weighted in an electrical balance.

3.8.2 Yield contributing parameter

The data of yield contributing characters of crop were calculated as follows:

I. Effective tillers hill^{-1} (no.)
II. Panicle length (cm)
III. Total number of grains
IV. 1000 grain weight (g)
V. Grain yield (g pot^{-1})
VI. Straw yield (g pot^{-1})

3.8.2.1 Effective tillers and non effectivetillers hill^{-1}

After harvesting of plant, tillers from each pot were observed, among them tillers with panicle and without panicle were counted and their average was calculated.

3.8.2.2 Panicle length

Measurement was taken from basal node of the rachis to apex of each panicle. Each observation was an average of 3 hills from each pot.

3.8.2.3 Filled and unfilled grains panicle^{-1}

Ten panicles were taken at random and the unfilled and filled grains per panicle were counted and averaged.

3.8.2.4 Grain yield

Grains from panicle from each pot were collected, dried from some days and then weighted.

3.8.2.5 Straw yield

After harvesting of plant, rice straw were collected and dried in a field. Dried straw were then weighted and their average was calculated.

3.8.2.6 1000 grain weight

The weight of 1000-grains from each pot was measured after sun drying by an electrical balance.

3.9 Statistical analysis

Statistix 10 was used for data analysis of this experiment.

Chapter IV

RESULT AND DISCUSSION

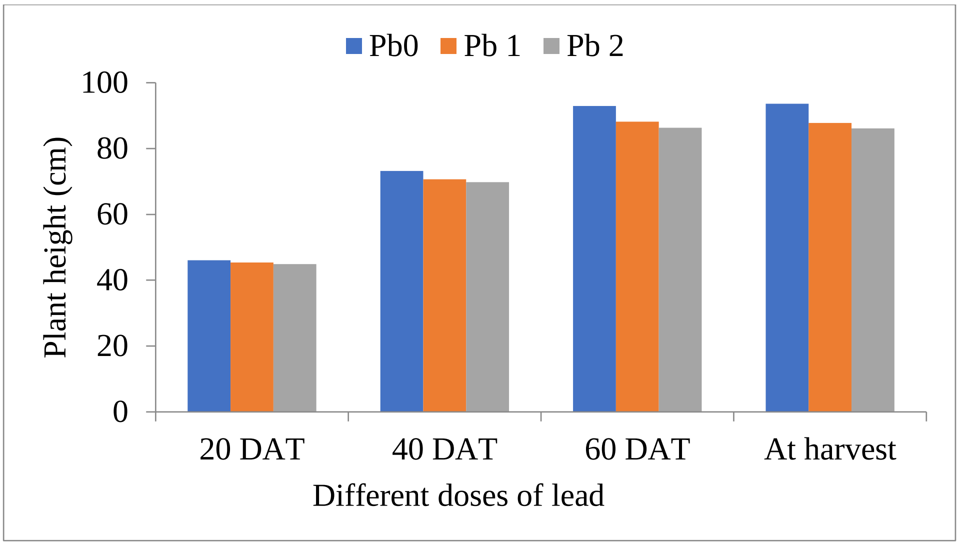
The present experiment was conducted to observe the impact of biochar on T.aman rice under lead(Pb) stress condition. Data on plant growth characters, yield contributing characters and concentration of Pb in rice grain were recorded to assess the trend of growth, development and yield of crops under Pb stress condition. The analysis of variance (ANOVA) of data is given in Appendix. The results have been presented and discussed under the following headings:

4.1 Growth Parameter

4.1.1 Plant height

4.1.1.1 Effect of lead (Pb)

Plant height was recorded at 40DAT, 60DAT and at harvest. There is a variation in plant height with the application of lead (Pb) (Figure 1). Due to the different level (0 $mgkg^{-1}$, 75 $mgkg^{-1}$ and 150 $mgkg^{-1}$ soil) of lead exposure, tallest plant height (45.12, 73.21, 92.97 and 93.63 cm at 20, 40, 60 DAT and at harvest, respectively) was observed with 0 $mgkg^{-1}$ soil of Pb. On the contrary, the shortest plant height (41.31, 69.80 , 86.32 and 86.12 cm at 20, 40, 60 DAT and at harvest, respectively) was observed with 150 $mgkg^{-1}$ soil of Pb. Exposure of Pb affects plant's morpho-physiological process such as growth and development of plants (Dogan *et al*., 2009).

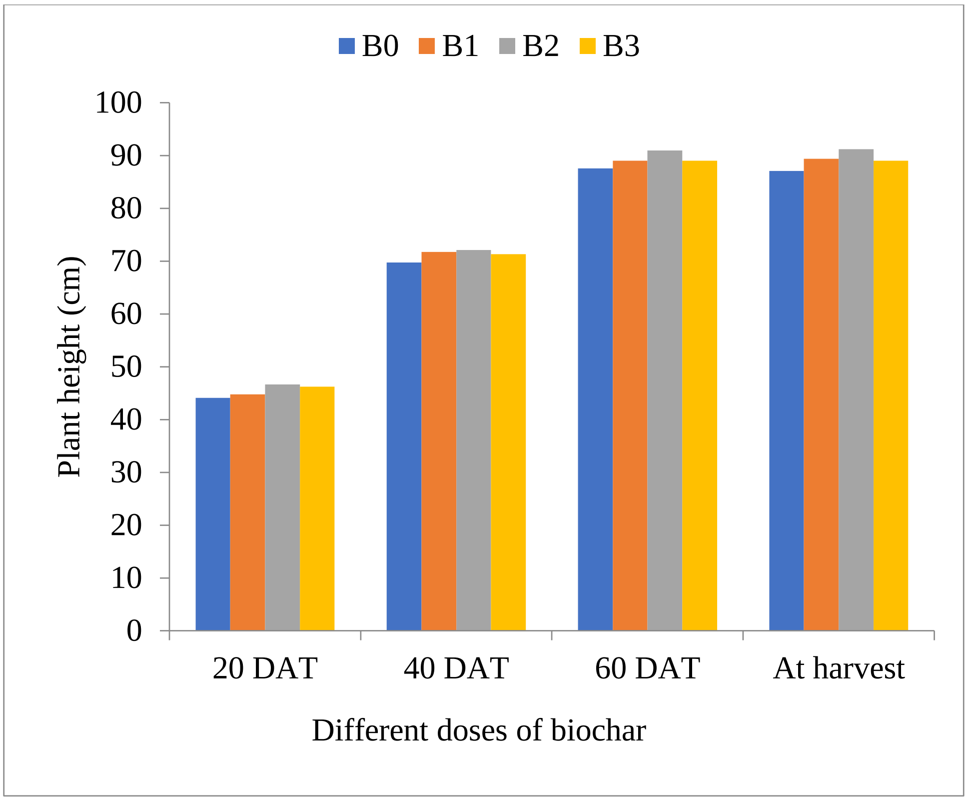


Here, Pb_0= $PbCl_2$ 0 mgkg^{-1} soil, Pb_1= $PbCl_2$75 mgkg^{-1} soil and Pb_2= $PbCl_2$150mgkg^{-1} soil

Figure 1. Effect of Lead on plant height of rice [LSD $_{(0.05)}$= NS,2.00, 2.02 and 1.77 at 20, 40, 60 DAT and at harvest, respectively]

4.1.1.2Effect of biochar

Application of different dose of biochar, showed significant variation in plant height (Figure 2). The highest plant height (48.52, 72.07, 90.97 and 91.22 cm at 20, 40, 60 DAT and at harvest, respectively) was recorded with 4 t ha^{-1}. Conversely shortest plant (42.12, 69.75, 87.54 and 87.08cm at 20, 40, 60 DAT and at harvest, respectively) was recorded with control. Biochar improves the soil fertility and plant growth by improving physical and chemical properties of soil and also increases the availability of useful nutrients (Houben *et al.*, 2013).



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 2. Effect of biochar on the plant height of rice [LSD $_{(0.05)}$= NS,NS, 2.34 and 2.04 at 20, 40, 60 DAT and at harvest, respectively]

4.1.1.3 Combination effect of lead (Pb) and biochar

Lead- induced stress resulted excessive accumulation of Pb in plant parts with phenotypic symptoms, which can be mitigate by the exogenous application of organic manure like biochar. External application of Pb and biochar interact and show different result (Table 1). In the interaction effect of Pb and biochar showed non significant differences on plant height (Table1). But among of them, highest result was found in the Pb_0B_2 treatment where 0 $mgkg^{-1}$ soil of Pb and 4 t ha^{-1} doses of biochar present.

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Table 2: Plant height of rice as influenced by combined effect of lead (Pb) and biochar

Treatments	Plant height (cm)			
	20 DAT	40 DAT	60 DAT	At harvest
Combined effect of Lead and Biochar				
Pb_0B_0	44.92	72.78	91.75	92.84
Pb_0B_1	45.38	72.88	93.03	93.92
Pb_0B_2	46.88	74.13	94.99	95.33
Pb_0B_3	46.92	73.08	92.09	92.42
Pb_1B_0	43.63	68.84	86.81	85.34
Pb_1B_1	44.63	72.08	87.74	87.65
Pb_1B_2	46.96	71.17	90.34	90.66
Pb_1B_3	46.42	70.50	87.64	87.49
Pb_2B_0	43.75	67.63	84.07	83.08
Pb_2B_1	44.29	70.34	86.25	86.58
Pb_2B_2	46.01	70.92	87.58	87.67
Pb_2B_3	45.38	70.33	87.39	87.17
$LSD_{0.5}$	NS	NS	NS	NS
CV (%)	7.07	8.89	13.16	12.76

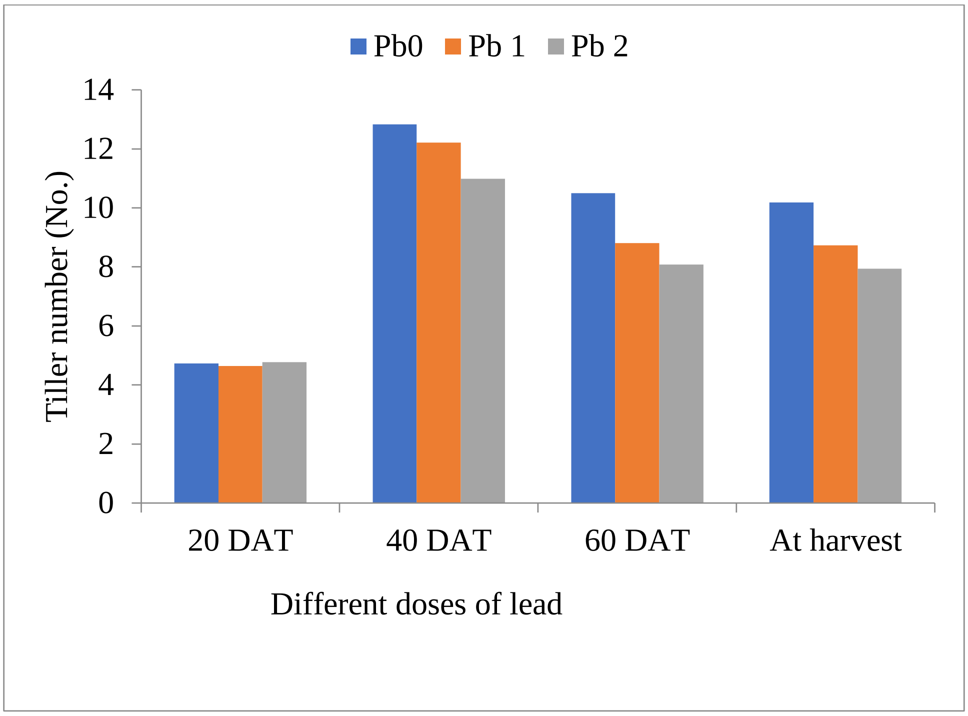
Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.1.2 Number of tillers hill^{-1}

4.1.2.1 Effect of lead (Pb)

When considering the tiller number, increasing Pb level in soil reduced the number of tillers. Tiller numbers were recorded at 20 DAT, 40DAT, 60DAT and at harvest. The highest tiller number (4.82, 12.83, 10.50 and 10.19 at 20, 40, 60 DAT and at harvest, respectively) (Figure3) was observed with 0 mgkg^{-1} soil of Pb (Pb_0). However, there was a significant reduction of tiller number (4.28, 10.99, 8.08 and 7.94 at 20, 40, 60 DAT and at harvest, respectively) for the application of 150 mgkg^{-1} soil of Pb (Pb_2). Liu *et al.*, (2010) showed that a certain concentration of Pb level in soil reduces plant biomass and earlier growth stage such as tillering number etc.

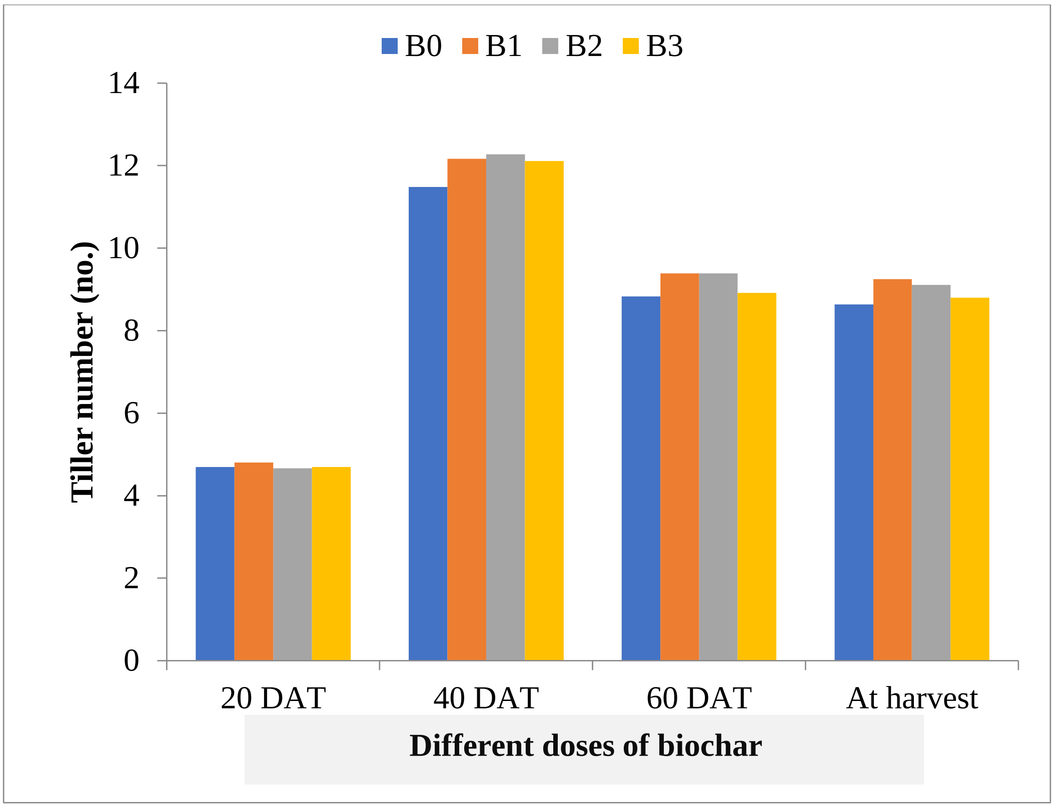


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150 $mgkg^{-1}$ soil

Figure 3. Effect of lead on the tiller number $hill^{-1}$of rice [LSD $_{(0.05)}$= NS,1.37, 1.12 and 0.97 at20, 40, 60 DAT and at harvest, respectively]

4.1.2.2 Effect of biochar

Biochar act as a growth developmental agent for the plant growth. Data was collected at 20, 40, 60DAT and at harvest. Even after using different doses of biochar, there has been no significant changes notice in tiller number of rice. Among of them 4.67, 12.28, 9.39, 9.11 tiller number found for 20, 40, 60 DAT and at harvest respectively in B_2 treatment where 4 t ha^{-1} doses of biochar present.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 4. Effect of biochar on tiller numberof rice [LSD $_{(0.05)}$= NS,NS, NS and NS at 20, 40, 60 DAT and at harvest, respectively]

4.1.2.3 Combination effect of lead (Pb) and biochar

In the interaction effect of Pb and biochar showed non significantdifferences on tiller number in rice. However Pb_0B_2 treatment gave highest result which were 4.50, 12.92, 10.92, 10.42 for 20, 40, 60 DAT, at harvest respectively (Table 3) . As a result, it was observed that biochar didn't mitigate Pb that much as expected.

Table 3: Tiller number hill^{-1} of rice as influenced by the combined effect of lead and biochar

Treatments	Tiller number (No.)			
	20 DAT	40 DAT	60 DAT	At harvest
Combined effect of Lead and Biochar				
Pb_0B_0	4.68	12.99	10.17	9.83
Pb_0B_1	4.92	12.84	10.84	10.40
Pb_0B_2	4.50	12.92	10.92	10.42
Pb_0B_3	4.84	12.59	10.08	10.00
Pb_1B_0	4.67	11.17	8.67	8.50
Pb_1B_1	4.83	12.59	9.00	8.92
Pb_1B_2	4.67	12.75	8.92	8.84
Pb_1B_3	4.42	12.34	8.67	8.67
Pb_2B_0	4.75	10.29	7.67	7.59
Pb_2B_1	4.67	11.09	8.34	8.33
Pb_2B_2	4.84	11.17	8.34	8.08
Pb_2B_3	4.84	11.42	8.00	7.74
$LSD_{0.5}$	NS	NS	NS	NS
CV (%)	14.98	15.96	17.13	15.10

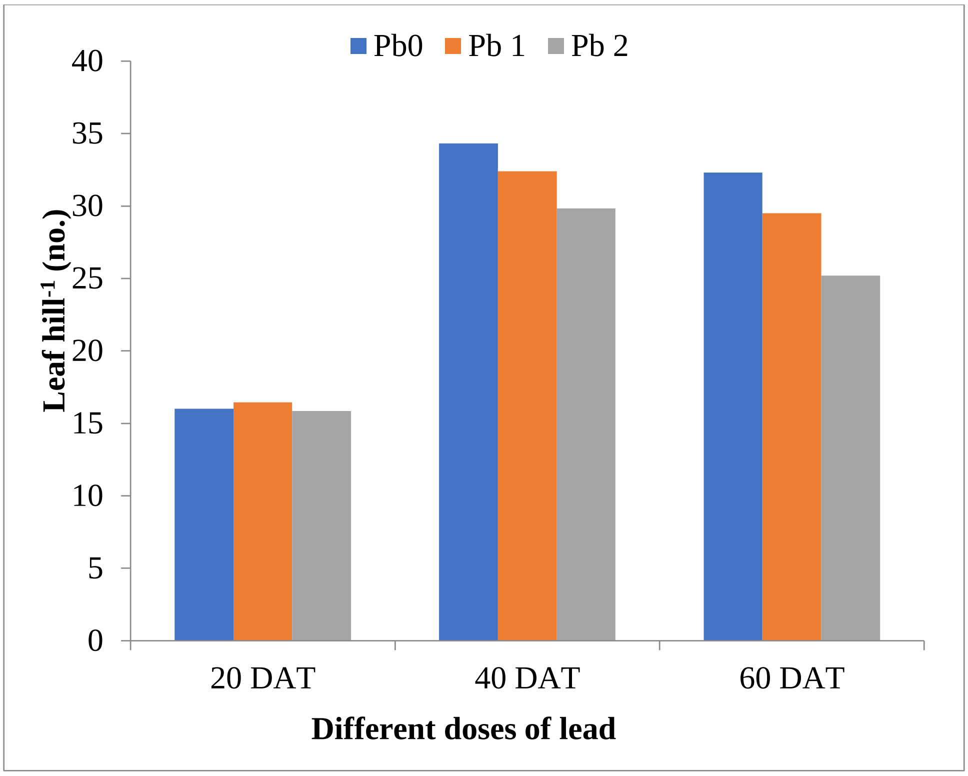
Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.1.3 No. of leaves hill^{-1}

4.1.3.1 Effect of lead (Pb)

Leaf number greatly affected by the exposure of different level of Pb (Figure5). Data was collected at 20 DAT, 40 DAT and 60 DAT. Thehighest leaf number (16.00, 31.31, and 32.31) was observed with 0 mgkg^{-1} soil of Pb (Pb_0). The lowest amount of leaves (15.85, 29.83 and 25.19) was recorded with 150 mgkg^{-1} soil of Pb(Pb_2).

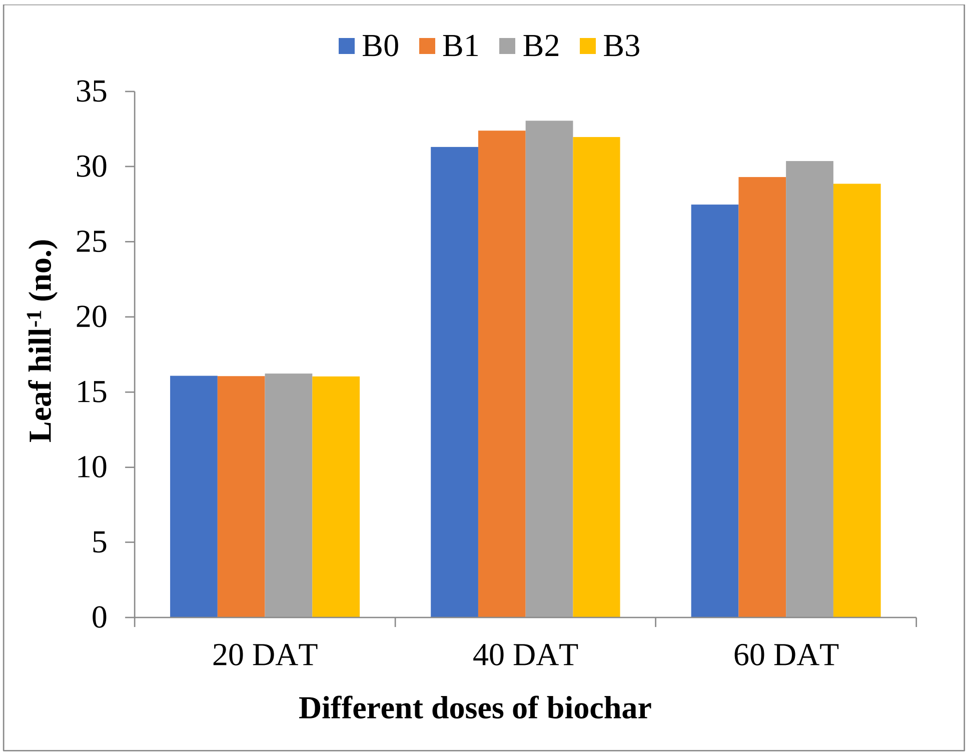


Here, Pb_0= $PbCl_2$ 0 mgkg^{-1} soil, Pb_1= $PbCl_2$75 mgkg^{-1} soil and Pb_2= $PbCl_2$150mgkg^{-1} soil

Figure 5. Effect of lead on leaf numberof rice [LSD $_{(0.05)}$= NS,1.37, 1.12 and 0.97 at 20, 40, 60 DAT and at harvest, respectively]

4.1.3.2 Effect of biochar

Application of biochar on rice plant didn't show any significant result on leaf number (Figure 6). But in B_2 (4 t ha^{-1}), highest leaf number found 16.22, 33.06, 30.36 for 20, 40 and 60 DAT respectively.



Here, B_0= Control, B_1= 2 t ha^{-1}of Biochar, B_2= 4 t ha^{-1}of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 6. Effect of biochar on leaf numberof rice [LSD $_{(0.05)}$= NS,NS, NS at 20, 40, 60 DAT respectively]

4.1.3.3 Combination effect of lead (Pb) and biochar

In the combination effect of Pb and biochar, there is no significant difference found in different treatments (Table 4). Almost similar results were observed in this interaction effect and therefore it can be said that biochar didn't effect the plant growth. However, highest leaf number found in Pb_0B_2(0 mgkg^{-1} of soil and 4 t ha^{-1} doses of biochar) treatment which is 16.25, 34.92, 33.08 for 20, 40 and 60 DAT, respectively.

Table 4: Leaf number and leaf area index of rice as influenced by the combination effect of lead and biochar

Treatments	Leaveshill^{-1} (No.)			Leaf area index	
	20 DAT	40 DAT	60 DAT	40 DAT	60 DAT
Combined effect of Lead and Biochar					
Pb_0B_0	15.83	33.74	31.59	0.067	0.073
Pb_0B_1	16.17	34.75	32.75	0.067	0.074
Pb_0B_2	16.25	34.92	33.09	0.068	0.074
Pb_0B_3	15.75	33.83	31.83	0.066	0.072
Pb_1B_0	16.67	31.83	27.92	0.061	0.064
Pb_1B_1	16.34	32.42	29.25	0.063	0.068
Pb_1B_2	16.83	33.09	31.17	0.065	0.069
Pb_1B_3	15.92	32.25	29.67	0.063	0.066
Pb_2B_0	15.75	28.33	22.92	0.052	0.053
Pb_2B_1	15.67	30.00	25.92	0.058	0.060
Pb_2B_2	15.59	31.17	26.84	0.061	0.063
Pb_2B_3	16.42	29.83	25.08	0.058	0.059
$LSD_{0.5}$	NS	NS	NS	NS	NS
CV (%)	15.94	7.24	9.41	4.93	5.13

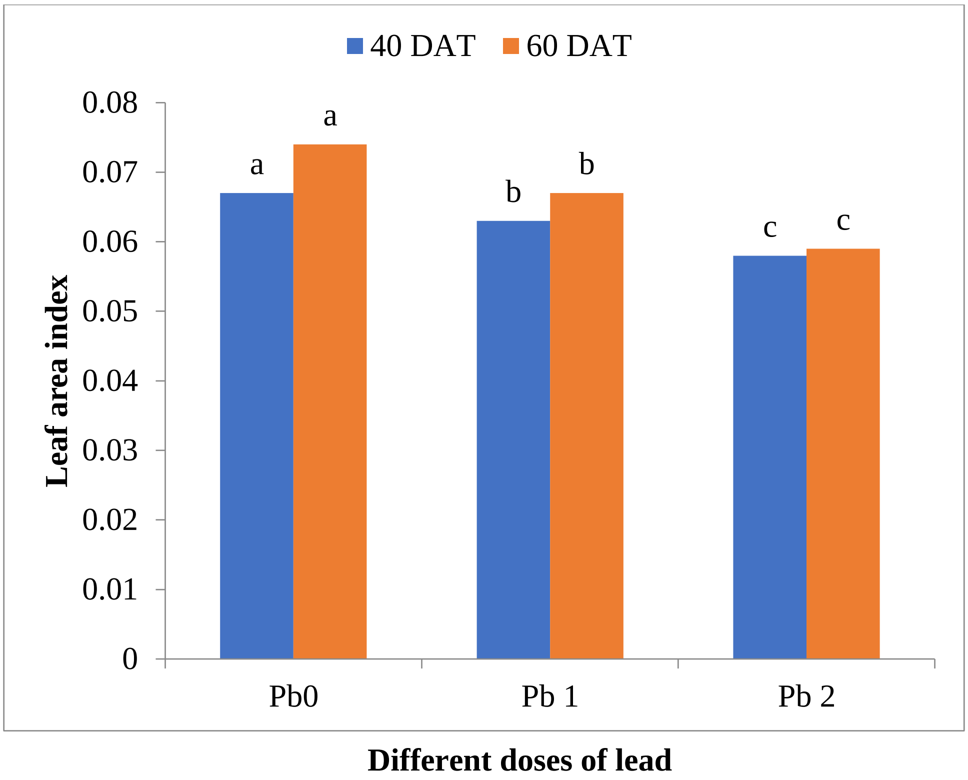
Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.1.4 Leaf area index

4.1.4.1 Effect of lead (Pb)

Leaf area of the plant reduced by the exposure of lead. At 40 and at 60DAT LAI data were recorded (Figure 7). Leaf area index greatly reduced (0.058 and 0.059) for the higher dose of Pb (150 mgkg^{-1} soil of Pb). However, LAI (0.063and 0.067) was observed under 75mgkg^{-1} soil of Pb .

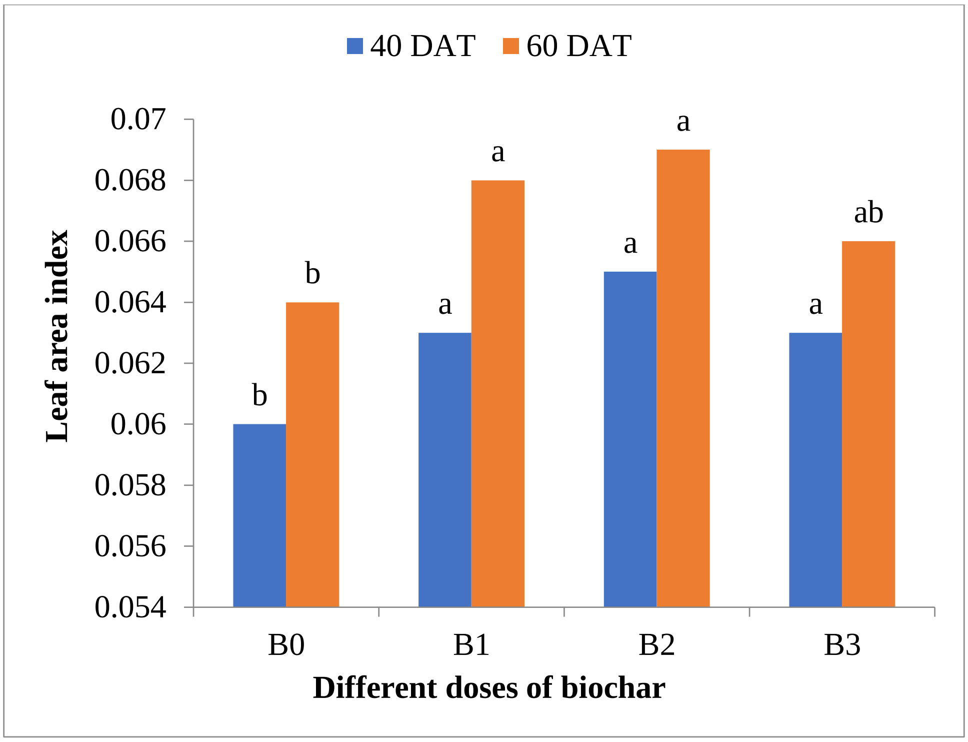


Here, Pb_0= $PbCl_2$ 0 mgkg^{-1} soil, Pb_1= $PbCl_2$75 mgkg^{-1} soil and Pb_2= $PbCl_2$150mgkg^{-1} soil

Figure 7. Effect of lead on leaf index of rice [LSD $_{(0.05)}$= 0.003, 0.005 at 40, 60 DAT, respectively]

4.1.4.2 Effect of biochar

Among the different doses of biochar, 4 t ha^{-1} gave good result in case of leaf area index. At 40DAT and 60DAT highest LAI (0.065 and 0.069, respectively) was recorded in B_2 (4 t ha^{-1} of Biochar) treatment and lowest LAI (0.060 and 0.064) was recorded in control condition (Figure 8).



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 8. Effect of biochar on leaf area indexof rice [$LSD_{(0.05)}$= 0.002, 0.003 at 40, 60 DAT respectively]

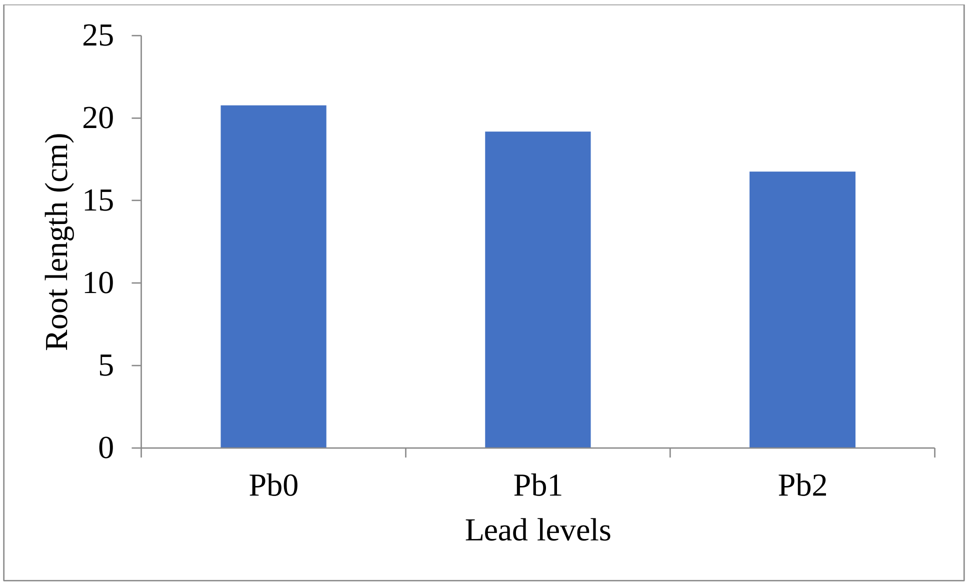
4.1.4.3 Combination of lead (Pb) and biochar

There was no significant difference found in the combination effect of Pb and biochar (Table 4). But numerically the highest LAI was recorded of 0.068 and 0.074 at 40 and 60 DAT in Pb_0B_2 treatment.

4.1.5 Root length

4.1.5.1 Effect of lead (Pb)

The longest root was recorded longer (20.78 cm) with 0 $mgkg^{-1}$ soil of Pb (Pb_0). The shortest root(16.75 cm) was found with 150 $mgkg^{-1}$ soil of Pb (Pb_2) (Figure9).Chatterjee *et al*., (2004) said thatPb exposure markedly reduced the biomass of seed, root, and shoot growth of the seedlings.

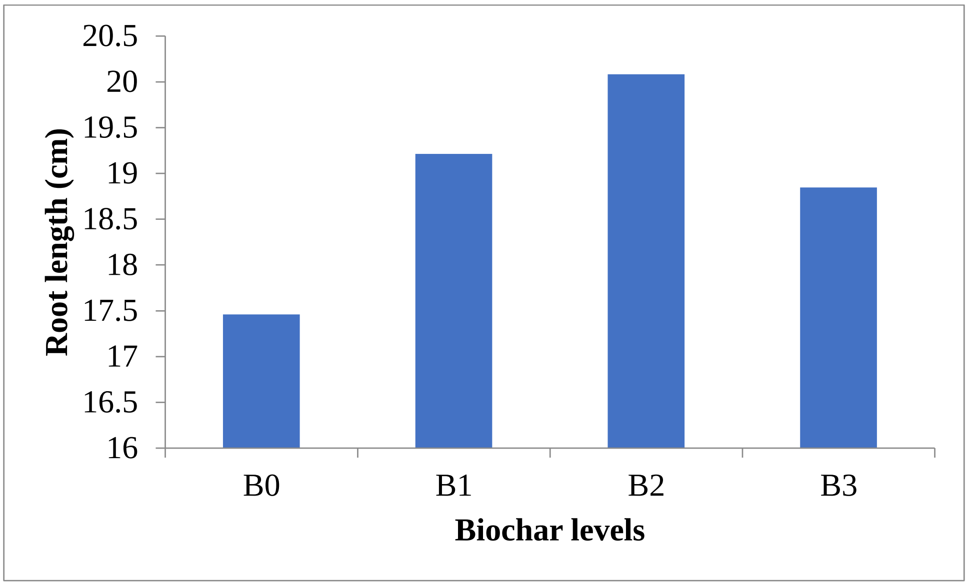


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150$mgkg^{-1}$ soil

Figure 9 Effect of lead on root length of rice at harvest[$LSD_{(0.05)}$= 1.52 at harvest]

4.1.5.2 Effect of biochar

The longest root (20.08cm) of root was found where 4 t ha^{-1} dose of biochar applied. On the other hand, the shortest root (17.46cm) was observed in control. B_2 gives longer root length which is apparently 15.03% higher from control.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 10: Effect of biochar on root length of rice at harvest[LSD $_{(0.05)}$= 2.02 at harvest respectively]

4.1.5.3 Combination effect of lead (Pb) and biochar

Biochar can be a way to minimize the Pb toxicity to a certain level. But in this experiment, there is no significant difference found in the interaction of Pb and biochar (Table 4). Among of them Pb_0B_2 showed numerically the highest result (21.50 cm).

Table 5: Root length and root weight of rice due to combined effect of lead and biochar

Treatments	Root length (cm)	Root weight(g)
Combined effect of Lead and Biochar		
Pb_0B_0	20.29	16.74
Pb_0B_1	20.75	16.99
Pb_0B_2	21.50	17.54
Pb_0B_3	20.58	15.83
Pb_1B_0	17.42	13.98
Pb_1B_1	19.81	16.01
Pb_1B_2	20.42	16.71
Pb_1B_3	19.04	15.84
Pb_2B_0	14.67	10.79
Pb_2B_1	17.09	12.78
Pb_2B_2	18.33	14.61
Pb_2B_3	16.92	12.79
$LSD_{0.5}$	NS	NS
CV (%)	8.88	10.92

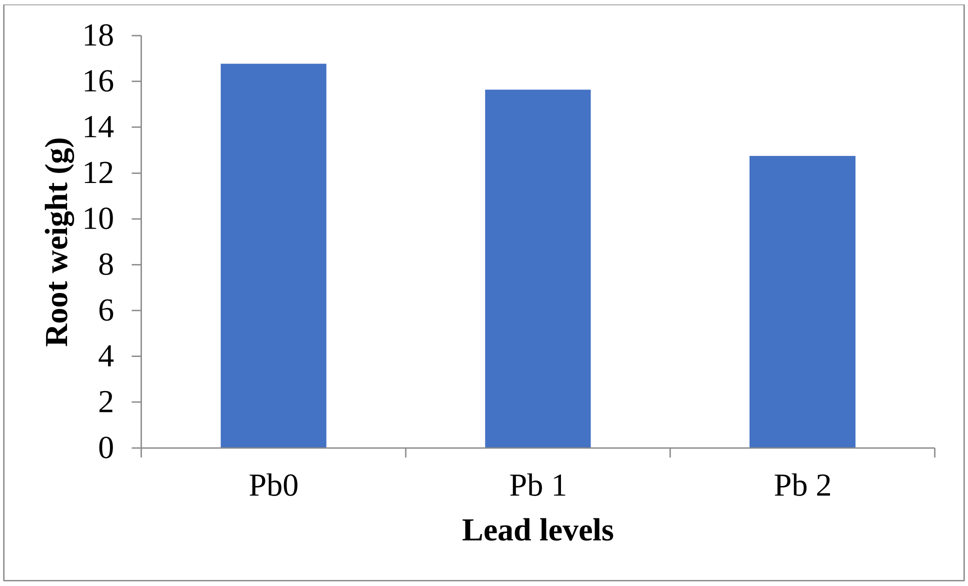
Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.1.6 Root weight

4.1.6.1 Effect of lead (Pb)

Lead affect the dry matter content of plant. The highest root weight (16.68 g) was recorded with 0 mgkg^{-1} soil of Pb (Pb_0) and the lowest root weight (12.74g) was recorded with 150 mgkg^{-1} soil of Pb (Pb_2) (Figure 11).

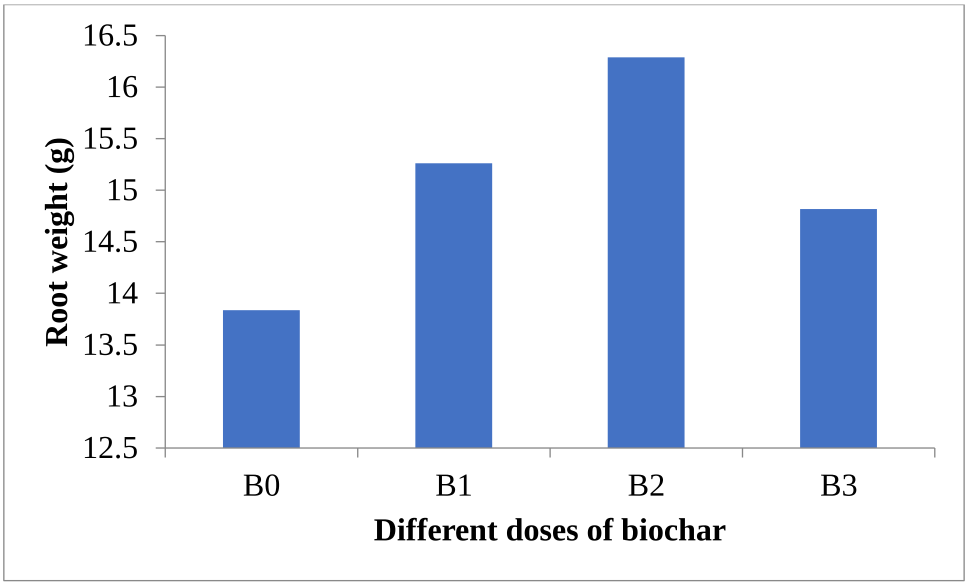


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150$mgkg^{-1}$ soil

Figure 11: Effect of lead on root weight of rice at harvest[LSD $_{(0.05)}$= 1.49 at harvest]

4.1.6.2 Effect of biochar

Biochar have the ability to impact on plant growth paameters and it was clearly seen in this experiment. The highest root weight (16.28g) was recorded with 4 t ha^{-1} of biochar dose. On the contrary, the lowest root weight (13.84g) was recorded in control condition.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 12 Effect of biochar on root weight of rice at harvest[LSD $_{(0.05)}$= 1.98 at harvest respectively]

4.1.6.3 Combination effect of lead (Pb) and biochar

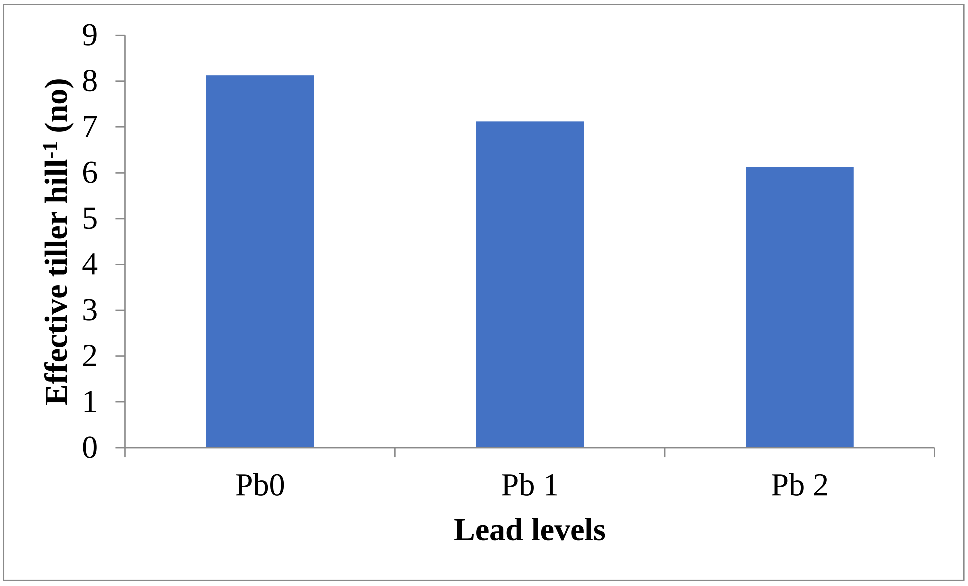
In the interaction effect of Pb and biochar showed non significant differences on root weight of rice (Table 5). 17.54 g root weight found in Pb_0B_2 treatments which is the highest in among of them.

4.2 Yield parameter

4.2.1 Effective tillers hill^{-1}

4.2.1.1 Effect of lead (Pb)

Highest number of effective tillers (8.13) found in Pb_0 (0 $mgkg^{-1}$ soil of Pb) and lowest number of effective tillers (6.13) was recorded in Pb_2 (150 $mgkg^{-1}$ soil of Pb)treatment which gives 24.62% lower tiller number from control.

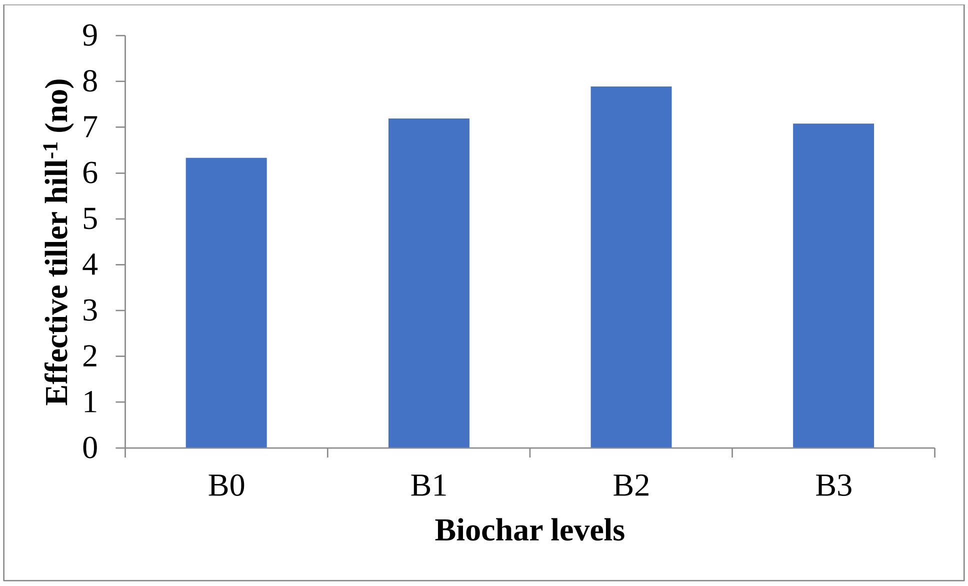


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150$mgkg^{-1}$ soil

Figure 13 Effect of lead on effective tillershill^{-1} (No.) of riceat harvest [$LSD_{(0.05)}$= 0.98 at harvest]

4.2.1.2 Effect of biochar

Highest number of effective tillers (7.89) was recorded in 4 t ha^{-1} dose of biochar and lowest number of effective tillers (6.33) was found in control condition. There is no significant variation found among the different doses of biochar.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 14: Effect of biochar on effective tillershill^{-1} (No.)of rice[$LSD_{(0.05)}$= 1.31 at harvest respectively]

4.2.1.3 Combination effect of lead (Pb) and biochar

There is no significant differences found in the interaction of Pb and biochar but among of them Pb_0B_2 (0 $mgkg^{-1}$ soil of Pb and 4 t ha^{-1} doses of biochar) gave highest result.

Table 6: Combined effect of Pb and Biochar on effective tiller hill^{-1}, panicle length, total grain hill^{-1}, 1000-grain weight of rice

Treatments	Effective tiller hill^{-1} (No.)	Panicle length (cm)	Total grain hill^{-1} (no.)	1000-grain wt. (g)
Combined effect of Lead and Biochar				
Pb_0B_0	7.67	19.56	313.41	20.84
Pb_0B_1	8.09	19.66	322.16	20.93
Pb_0B_2	9.00	20.05	334.75	21.44
Pb_0B_3	7.75	19.42	318.50	20.97
Pb_1B_0	6.25	19.36	289.00	19.15
Pb_1B_1	7.25	19.67	296.50	20.28
Pb_1B_2	7.67	19.39	306.75	20.73
Pb_1B_3	7.33	19.71	297.50	20.37
Pb_2B_0	5.09	19.15	247.75	17.57
Pb_2B_1	6.25	19.51	256.75	18.76
Pb_2B_2	7.00	19.56	262.25	19.68
Pb_2B_3	6.17	19.61	256.00	18.91
$LSD_{0.5}$	NS	NS	NS	NS
CV (%)	15.25	4.31	3.09	3.25

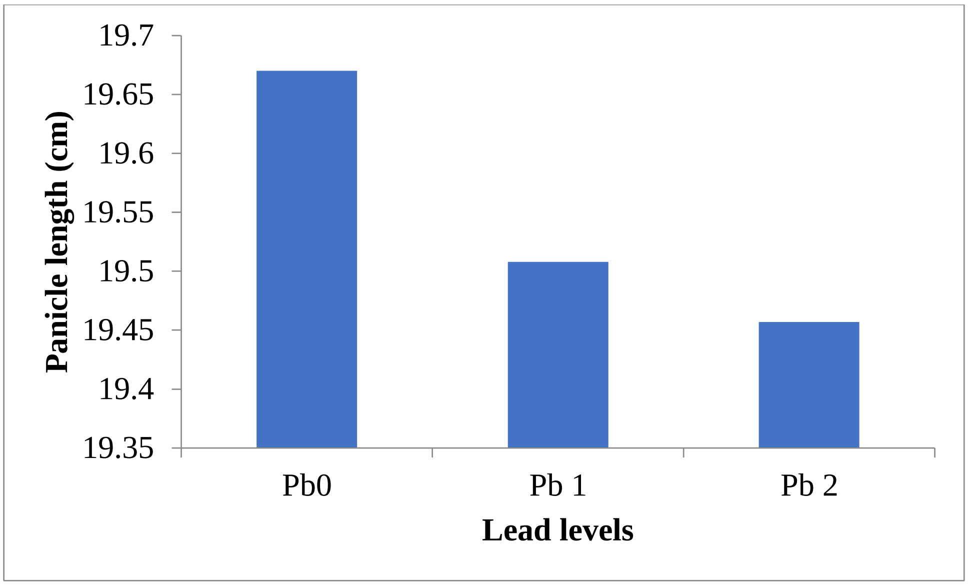
Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.2.2 Panicle length

4.2.2.1 Effect of lead (Pb)

Exposure of Pb have no effect on panicle length of T. aman rice (Figure 15). Application of Pb can reduce the panicle length of rice. But in this experiment Pb_0, Pb_1, Pb_2 gives comparatively similar results which are 19.67, 19.51, 19.46 cm,respectively.

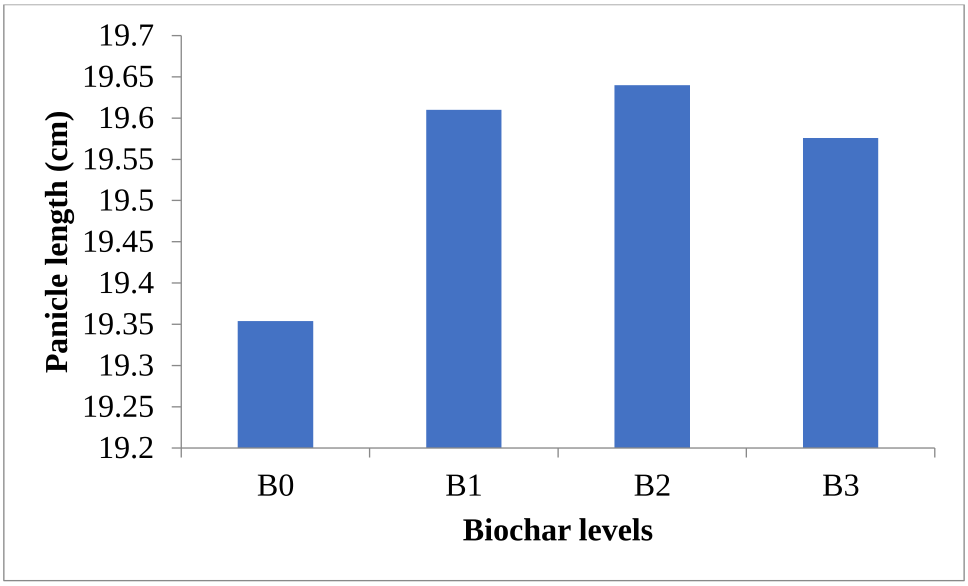


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150$mgkg^{-1}$ soil

Figure 15: Effect of lead on panicle length of rice at harvest time[$LSD_{(0.05)}$= NS at harvest]

4.2.2.2 Effect of biochar

There was no variation noticed in panicle length for the application of different doses of biochar (Figure 16). Panicle length19.35, 19.61, 19.64, 19.58 cm for 0, 2, 4, 6 t ha^{-1} doses of biochar which are comparatively same.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 16 Effect of biochar on panicle length of rice at harvest time[LSD $_{(0.05)}$= NS at harvest]

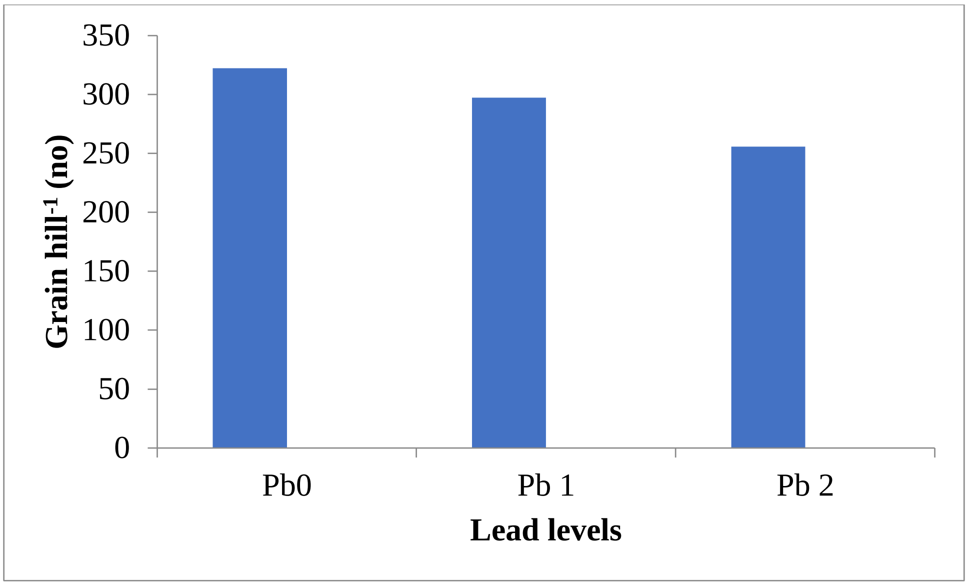
4.2.2.3 Combination effect of lead (Pb) and biochar

Interaction of Pb and biochar have no significant effect on on panicle length of rice (Table 6).But among of them Pb_0B_2 showed highest panicle length (20.05 cm).

4.2.3 Total grains $hill^{-1}$

4.2.3.1 Effect of lead (Pb)

The highest number of grains per pot (322.21) was recorded in 0 $mgkg^{-1}$ soil of Pb and the lowest number of grains was recorded (255.69) in 150 $mgkg^{-1}$ soil of Pb.

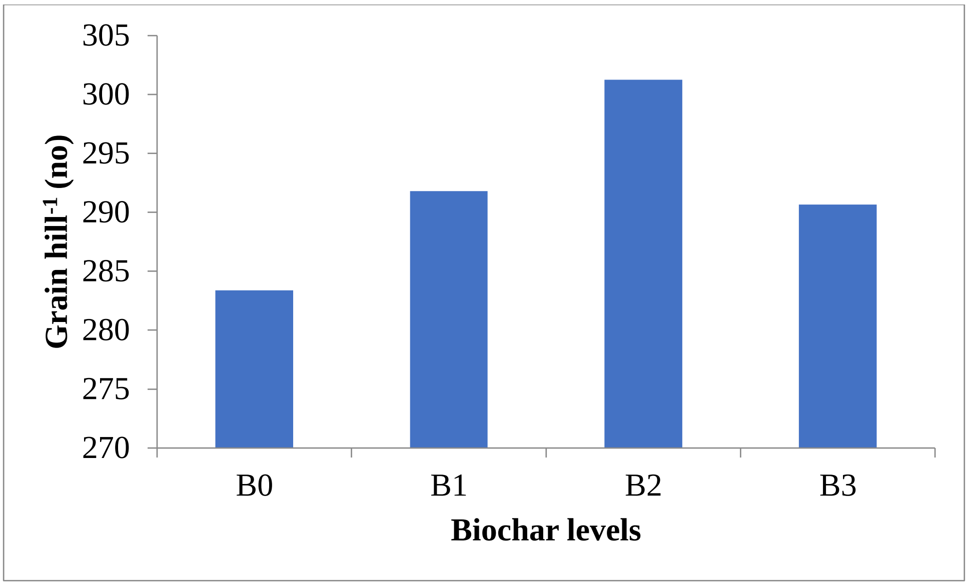


Here, Pb_0= $PbCl_2$ 0 mgkg^{-1} soil, Pb_1= $PbCl_2$75 mgkg^{-1} soil and Pb_2= $PbCl_2$150mgkg^{-1} soil

Figure 17 Effect of lead on number of grains hill^{-1} of rice[LSD $_{(0.05)}$= 8.17 at harvest]

4.2.3.2 Effect of biochar

Application of biochar increased the number of grain in rice. The highest number of total grain per pot (301.25) was recorded by the 4 t ha^{-1} dose of biochar and the lowest number of total grain per pot was found in 0 t ha^{-1}dose of biochar.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 18: Effect of biochar on number of grain of rice[LSD $_{(0.05)}$= 10.85 at harvest respectively]

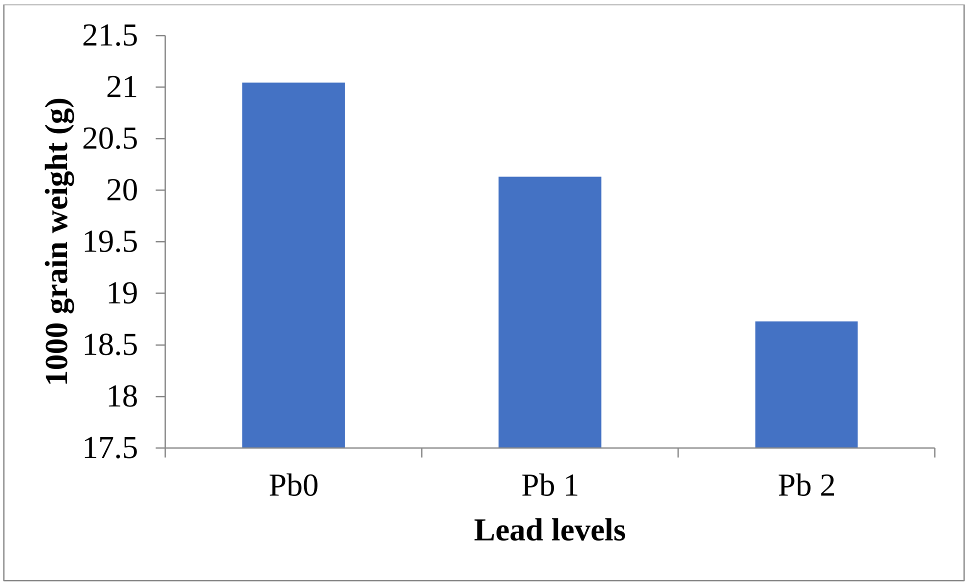
4.2.3.3 Combination effect of lead (Pb) and biochar

There was no significant variation found in grain number for the the combination of Pb and biochar.. But among of them Pb_0B_2 give the highest number of grain per pot (334.75).

4.2.4 1000-grain weight

4.2.4.1 Effect of 1000-grain weight

Lead (Pb) adversely affect the grain yield, as in this experiment, the highest dose of Pb (150 $mgkg^{-1}$ soil of Pb) resulted the lowest 1000-grain weight which is 18.73g. In an opposite way the highest 1000-grain weight (21.04 g) was recorded in control condition.

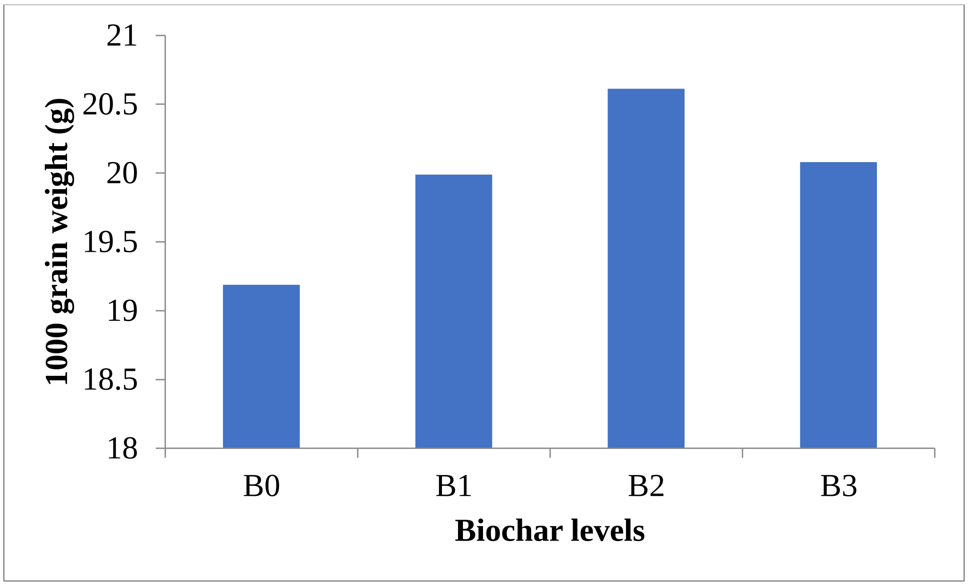


Here, Pb_0= $PbCl_2$ 0 mgkg^{-1} soil, Pb_1= $PbCl_2$75 mgkg^{-1} soil and Pb_2= $PbCl_2$150mgkg^{-1} soil

Figure19: Effect of lead on 1000 grain weight of rice[LSD $_{(0.05)}$= 0.59 at harvest]

4.2.4.2 Effect of biochar

The highest 1000-grain weight (20.61g) was recorded in 4 t ha^{-1} doses of biochar and the lowest amount of 1000-grain weight (19.19 g) was recorded in control condition.



Here, B_0= Control, B_1= 2 t ha^{-1}of Biochar, B_2= 4 t ha^{-1}of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 20: Effect of biochar on 1000 grain weight of rice[LSD $_{(0.05)}$= 0.78 at harvest respectively]

4.2.4.3 Combination of lead (Pb) and biochar

The interaction of Pb and biochar showed non significant difference on 1000-grain weight (Table 6). But among of them Pb_0B_2 (0 $mgkg^{-1}$ soil of Pb and 4 t ha^{-1} dose of biochar) treatment give the highest result.

4.2.5 Grain yield $hill^{-1}$

4.2.5.1 Effect of lead (Pb)

Exposure of higher amount of Pb create adverse condition for plant.The highest grain yield per pot (20.33 g) was observed in 0 $mgkg^{-1}$ soil of Pb, whereas the lowest grain yield per pot (14.31g) was recorded in Pb_2 (150 $mgkg^{-1}$ soil of Pb). The lowest grain yield was recorded for the exogenous application of 150 $mgkg^{-1}$ soil of Pb because it decreased the yield contributing parameter such as effective tillers, 1000-grain weight, number of grain. Liu *et al.*, (2010) showed that a certain concentration of Pb level inhibits plant growth and also reduce yield.

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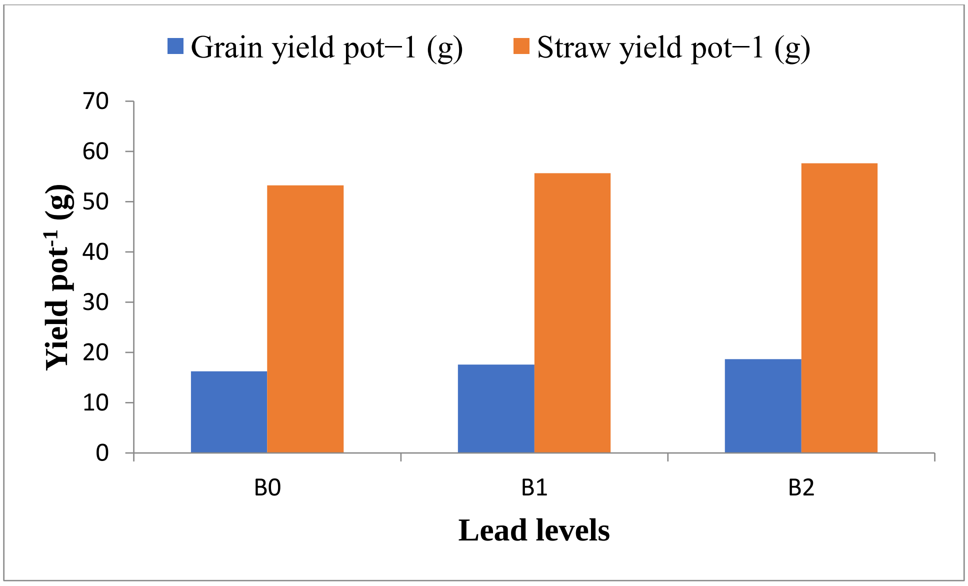
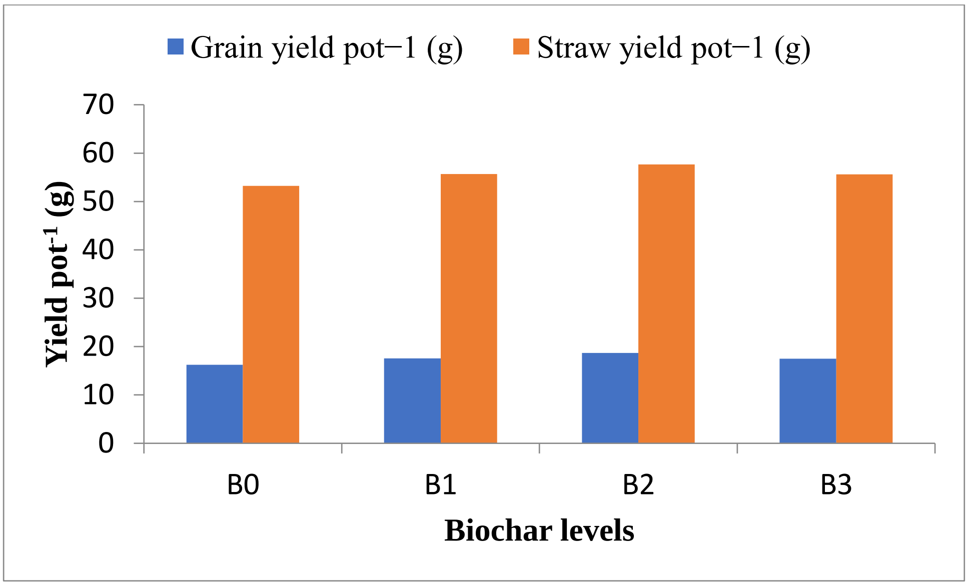


Here, Pb_0= $PbCl_2$ 0 $mgkg^{-1}$ soil, Pb_1= $PbCl_2$75 $mgkg^{-1}$ soil and Pb_2= $PbCl_2$150$mgkg^{-1}$ soil

Figure 21: Effect of lead on grain yield pot^{-1} (g)of rice[LSD $_{(0.05)}$= 0.74 at harvest]

4.2.5.2 Effect of biochar

The Highest grain yield per pot (18.70g) was recorded for the application of biochar 4 t ha[1] (B_2). The lowest grain yield per pot(16.25g) was recorded in control condition. Control condition gave lowest yield compared with all doses of biochar as yield contributing parameter effective tiller and 1000-grain weight also lowest in control condition.



Here, B_0= Control, B_1= 2 t $ha^{-1}$of Biochar, B_2= 4 t $ha^{-1}$of Biochar and B_3= 6 t ha^{-1} of Biochar

Figure 22: Effect of biocharon grainyield pot^{-1} (g)of rice[LSD $_{(0.05)}$= 0.99 at harvest respectively]

4.2.5.3 Combination effect of lead (Pb) and biochar

There is no significant difference observed in the interaction of Pb and biochar treatments for grain yield of rice (Table 7). But among of them Pb_0B_2 treatments gives 21.54g grain yield which is the highest one.

Table 7: Grain yield and straw yield of T. aman rice as affected by the interaction of lead and biochar

Treatments	Grain yield pot^{-1} (g)	Straw yield pot^{-1} (g)
Combined effect of Lead and Biochar		
Pb_0B_0	19.59	57.71
Pb_0B_1	20.21	58.76
Pb_0B_2	21.54	60.42
Pb_0B_3	19.97	57.85
Pb_1B_0	16.36	54.28
Pb_1B_1	18.03	56.42
Pb_1B_2	19.09	57.65
Pb_1B_3	18.08	56.71
Pb_2B_0	12.82	47.72
Pb_2B_1	14.45	51.92
Pb_2B_2	15.48	54.97
Pb_2B_3	14.51	52.34
$LSD_{0.5}$	NS	NS
CV (%)	4.69	5.06

Pb_0= $PbCl_2$ 0 mgkg^{-1} soil
Pb_1= $PbCl_2$75 mgkg^{-1} soil
Pb_2= $PbCl_2$150 mgkg^{-1} soil

B_0= Control
B_1=2 t ha^{-1}Biochar
B_2= 4 t ha^{-1}Biochar
B_3= 6 t ha^{-1}Biochar

4.2.6 Straw yield

4.2.6.1 Effect of lead (Pb)

A good straw yield per pot (58.68g) was recorded in Pb_0 (0 mgkg^{-1} soil of Pb) and lowest straw yield per pot (51.74g) was recorded in Pb_2 (150mgkg^{-1} soil of Pb) treatment.

4.2.6.2 Effect of biochar

Highest straw yield per pot (57.68g) was found in 4 t ha^{-1} dose of biochar and lowest straw yield per pot was recorded in control condition which is 53.23g.

4.2.6.3 Combination effect of lead (Pb) and biochar

In the interaction effect of Pb and biochar showed non significant results on straw yield (Table 7). But among of them Pb_0B_2 treatment give the highest result which is 60.422.

Chapter V

SUMMARY AND CONCLUSION

Lead is the major inorganic contaminant that is being used since antiquity. Its presence in soil or atmosphere is harmful for living beings. In soil, it forms complexes with soil elements, interferes with plant-soil-environment relationships. It is unique in its behavior, mobility, form, and solubility within the soil and bioavailability to the plants. It must be mitigate for contaminants free rice cultivation.

The pot experiment was assessed at the Agronomy net house of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period in July 2018 to December 2018 to understand the role of biochar under lead stress condition.Inmy study I observed that exposure of Pb decreased plant growth such has plant height, tiller number, number of leaves and root length etc.At 40, 60 and at harvest the longest plant (73.21 , 92.97 and 93.63 cm, respectively) was observed in control condition (Pb_0) and the shortest plant (69.80, 86.32 and 86.12 cm, respectively)was observed in 150 mg kg^{-1} soil of Pb (Pb_2). In the same way the lowest number of tillers (10.99, 8.08, 7.93 at 40, 60 DAT and at harvest, respectively) was recorded in 150 mg kg^{-1} soil of Pb. The lowest number of leaves and at 40 (29.83) and 60 DAT (25.20)observed in $Pb_{2.}$ Leaf area index also highest in control conditions and lowest in severe Pb stress condition. At harvest root length was highest in control condition (20.78 cm) lowest (16.75 cm) in Pb_2. Similarly the highest root weight (16.77g) was recorded in Pb_0 and lowest root weight (12.74g) was recorded in Pb_2. Yield contributing parameter also showed similar trend in Pb stress conditions. The highest number of effective tillers (8.12) was recorded in Pb_0 and the lowest number of effective tiller (6.12) was recorded in Pb_2. The number of grain per pot was lowest (255.69) in Pb_2. Thousand grain weight also hihest (21.04g) in Pb_0 and lowest(18.73g) in Pb_2. The higestr grain yield per pot (20.33g) was observed in Pb_0 and thelowest grain yield per pot (14.31g) was recorded in Pb_2. A good Straw yield (58.68g) was recorded in Pb_0 and the lowest straw yield (51.73g) was recorded in Pb_2. In case of biochar application , at 20, 40, 60 and at harvest, the highest plant height (46.62, 72.06, 90.97 and 91.22 cm) was recorded in B_2(4 t ha^{-1}) and shortest plant (44.09cm, 69.74cm, 87.54 cm and 87.08cm) was recorded in control condition (B_0).. At 40 and 60 DAT the highest LAI (0.065 and 0.069, respectively) was recorded in

$B_2$and the lowest LAI (0.060 and 0.064, respectively) was recorded in B_0. The longest (20.08cm) root was found in B_2 and the shortestroot (17.46cm) was observed in B_0. . The highest number of effective tillers (7.89) was observed in B_2. On the other hand, the lowest number of effective tillers (6.33) was recorded in B_0 treatments. The highest number of total grain per hill is found in B_2 (301.25) and the lowest number of total grain per hill is recorded in B_0 (283.39). Application of 4 t $ha^{-1}$of biochar (B_2)gavethe highest 1000 grain weight which is 20.61g and B_0 gives the lowest 1000 grain wt (19.19). The highest grain yieldper pot was found in B_2 (18.70) and the lowest grain yield per pot was found in B_0 (16.25 g).

In case of interactions effect of Pb and biochar showed non significant differences on growth and yield contributing parameters. From the above discussion it might be concluded that, exposure of Pb decreased growth and yield of T. aman rice (BRRI dhan62). The growth and yield of rice decreased with increasing the doses of Pb. On the other hand, Biochar had positive impact on growth and yield of rice in certain cases. The growth and yield of rice increased with increasing the dose of biochar upto a certain extent (4 t ha^{-1}). It was perceived that mitigation of Pb toxicity in rice plant by using biochar is not noticeable at all. In case of growth and yield parameter of rice, biochar can play a minor role. Biocharcannot be a reasonable approach to reduce the Pb stress in rice.

RECOMMENDATIONS

Heavy metal contaminated soil adversely affect the growth and development of plants which results in a reduction of crop productivity. Moreover, Lead (Pb) contaminated food is a serious threat to human health. Nevertheless, biochar can not to be an effective way to overcome the problem of Pb toxicity. So recommendations are asked to explore:

1. Biochar can notbe used for mitigation of Lead (Pb) toxicity in crop production.

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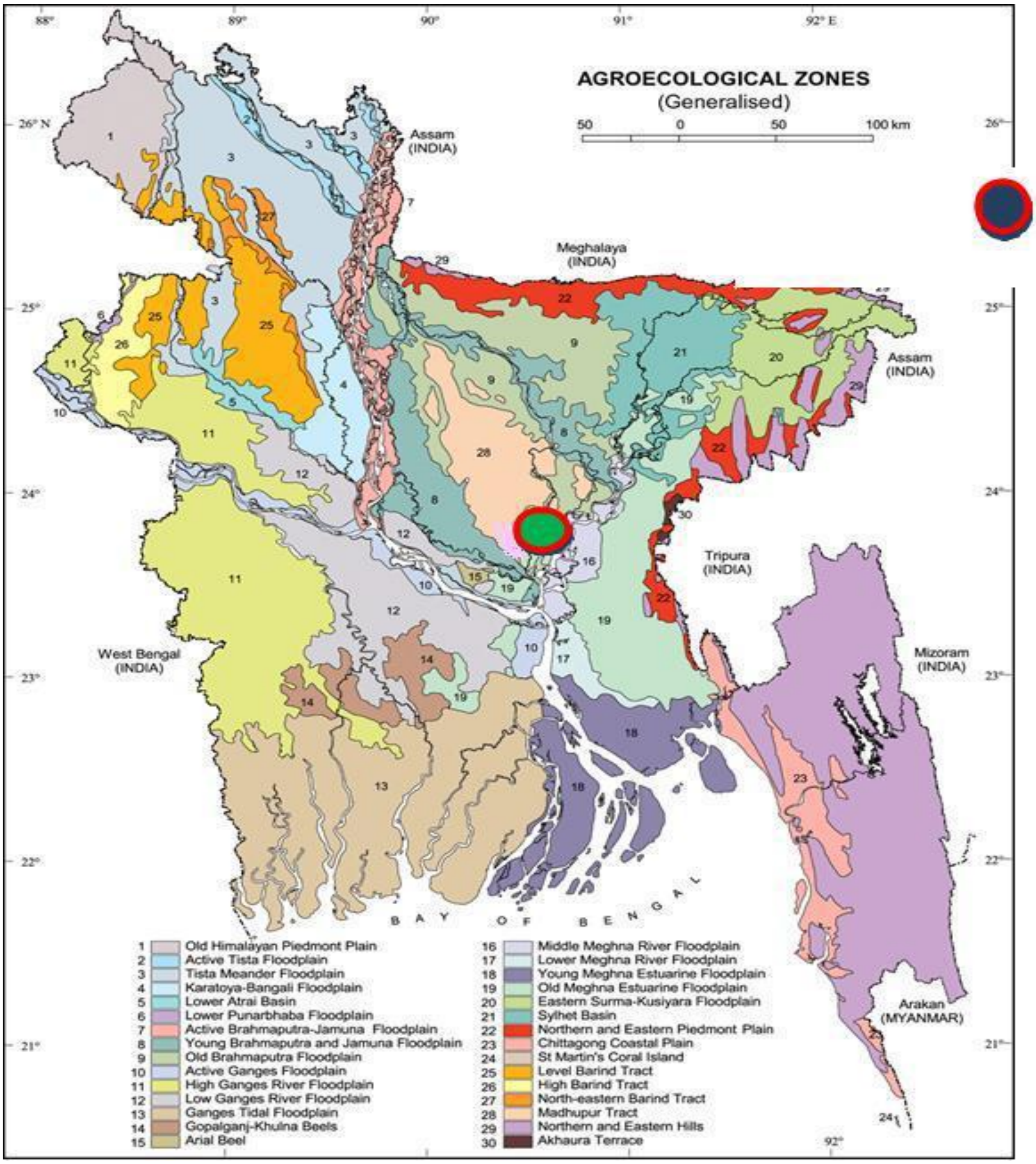
Appendices

Appendix Ⅰ.Map showing the experimental site under the study



Appendix II-Monthly average temperature, average relative humidity and total rainfall and average sunshine of the experimental site during the period from July- December, 2018

Month	Year	Monthly average air temperature (°C)			Average relative humidity (%)	Rainfall (mm)	Average sunshine (hours)
		Maximum	Minimum	Mean			
June	2018	23.2	35.5	29.4	78	312	5.4
July	2018	24.5	36.0	30.3	83	563	5.1
August	2018	23.5	36.0	29.8	81	319	5.0
Sep.	2018	24.4	34.5	29.5	81	279	4.4
Oct.	2018	25	32	28.5	79	175	6
Nov.	2018	21	30	25.5	65	35	8
Dec.	2018	15	29	22	74	15	9

Source: Bangladesh Meterological department (Climate and weather division), Aargaon, Dhaka-1212

Appendix III- Mean sum square values of the data for plant height at different days after transplanting

R$_1$

Source of VariatioN	**DF**	**Mean Sum square values of plant height**			
		20 DAT	40 DAT	60 DAT	At harvest
Replication	3	3.5897	2.4476	13.357	1.484
Lead (Pb)	2	5.42 NS	50.5339**	188.843 **	248.246 **
Biochar (B)	3	17.09 NS	12.8002 NS	23.688 *	34.497 **
Lead*Biochar (Pb*B)	6	0.47 NS	2.4919 NS	2.252 NS	4.353 NS
Error	33	10.3258	7.6837	7.938	6.063
Total	47				

Appendix IV-Mean sum square values of the data for tiller number at different days after transplanting

Source of Variation	DF	Mean Sum square values of tiller number			
		20 DAT	40 DAT	60 DAT	At harvest
Replication	3	0.39550	6.8795	0.5414	4.3928
Lead (Pb)	2	0.06258 NS	14.0697 *	24.5946 *	20.8824 **
Biochar (B)	3	0.04494 NS	1.5286 NS	1.0743 NS	0.9390 NS
Lead*Biochar (Pb*B)	6	0.11777 NS	0.7944 NS	0.1079 NS	0.0340 NS
Error	33	0.49872	3.6727	2.4481	1.8271
Total	47				

Appendix V- Mean sum square values of the data for leaf number at different days after transplanting

Source of Variation	DF	Mean Sum square values of leaf number		
		20 DAT	40 DAT	60 DAT
Replication	3	19.1613	10.4751	12.245
Lead (Pb)	2	1.4864	80.7319 **	205.99 **
Biochar (B)	3	0.0881	6.5059 NS	17.19 NS
Lead*Biochar (Pb*B)	6	0.6924	0.7398 NS	1.62 NS
Error	33	6.5825	5.4226	7.441
Total	47			

Appendix VI- Mean sum square values of the data for leaf area index at different days after transplanting

Source of Variation	DF	Mean Sum square values of leaf area index	
		40 DAT	60 DAT
Replication	3	3.472	1.456
Lead (Pb)	2	3.777 **	8.286 **
Biochar (B)	3	4.281 **	5.872 **
Lead*Biochar (Pb*B)	6	1.541 NS	1.528 NS
Error	33	9.533 NS	1.166
Total	47		

Appendix VII- Mean sum square values of the data for root length and root weightat harvest

Source of Variation	DF	Mean Sum square values of root	
		Root length	Root weight
Replication	3	0.7228	1.6018
Lead (Pb)	2	65.88 **	69.0642 **
Biochar (B)	3	14.31 **	12.4135 **
Lead*Biochar (Pb*B)	6	1.39 NS	2.3996 NS
Error	33	2.8160	2.7034
Total	47		

AppendixVIII- Mean sum square values of the data for yield contributing parameters

Source of Variation	DF	Effective tiller hill^{-1}	Panicle length	Total grain hill^{-1}	1000-grain wt.
Replication	3	2.16	0.93576	214.7	1.0440
Lead (Pb)	2	16.00 **	0.198 NS	18084.1 **	21.78 **
Biochar (B)	3	4.86 **	0.20 NS	645.4 **	4.16 **
Lead*Biochar (Pb*B)	6	0.29 NS	0.22 NS	20.3 NS	0.51 NS
Error	33	1.18	0.71	81.4	0.42
Total	47				

Appendix IX-Mean sum square values of the data for grain and straw yield of rice

Source of Variation	DF	Mean Sum square values of grain and straw yield	
		Grain yield	Straw yield
Replication	3	0.253	15.906
Lead (Pb)	2	146.419 **	199.042 **
Biochar (B)	3	12.030 **	39.751 **
Lead*Biochar (Pb*B)	6	0.433 NS	5.318 NS
Error	33	0.674	7.908
Total	47		